

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> :

G06F 19/00

A2

(11) International Publication Number:

WO 00/70528

(43) International Publication Date: 23 November 2000 (23.11.00)

(21) International Application Number: PCT/US00/13154

(22) International Filing Date: 12 May 2000 (12.05.00)

(30) Priority Data:

09/310,879	14 May 1999 (14.05.99)	US
09/311,890	14 May 1999 (14.05.99)	US
09/311,996	14 May 1999 (14.05.99)	US
60/134,104	14 May 1999 (14.05.99)	US
Not furnished	26 April 2000 (26.04.00)	US

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(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

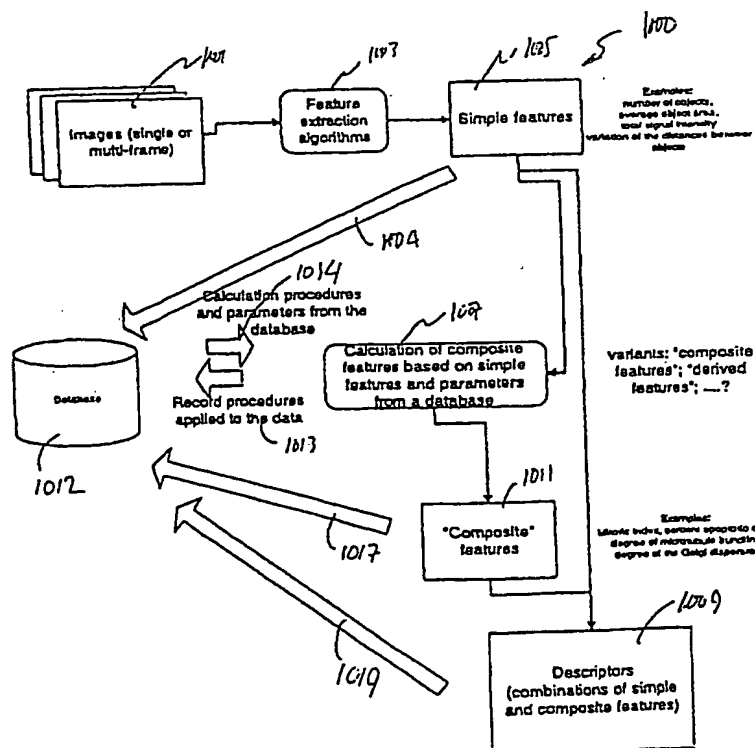
**Published**

*Without international search report and to be republished upon receipt of that report.*

(54) Title: METHOD AND APPARATUS FOR PREDICTIVE CELLULAR BIOINFORMATICS

(57) Abstract

Techniques for using information technology in therapeutics or drug discovery. In an exemplary embodiment, techniques for determining information about the properties of substances based upon information about structure of living or non-living cells exposed to substances are provided. A method according to the present invention enables researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database. The present invention further teaches a system for acquiring knowledge from cellular information. The system has a database 1012 comprising a database management module ("DBMS"). The system also has a variety of modules, including a population module coupled to the DBMS for categorizing and storing a plurality of features (e.g., cell size, distance between cells, cell population, cell type) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.



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**PATENT APPLICATION**  
**METHOD AND APPARATUS FOR**  
**PREDICTIVE CELLULAR BIOINFORMATICS**  
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10 computer codes, which may be used to implement aspects of the present invention. Assignee of the present invention reserves all rights with respect to these codes and provides notice herein. Notice is hereby given © Cytokinetics, Inc. 1999.

**BACKGROUND OF THE INVENTION**

The present invention provides techniques for information  
15 management using a database platform. More particularly, the present invention provides a system including computer code that couples to a database device. The system provides for image capturing of living, dead, or fixed cells or cell fractions used to identify information about substances used on the cells or information about the cells themselves. Accordingly, the present invention can enable researchers and  
20 scientists to identify promising candidates in the search for new and better medicines, for example, in drug discovery and development. The principles enumerated herein may, with equal facility, be applied to other applications, including but not limited to use in environmental applications such as determining chemical toxicities and other non-pharmaceutical toxicology uses.

25 For a long time, researchers in the pharmaceutical field have sought for better ways of searching for substances possessing properties that make them suitable as medicines. In the early days, researchers generally relied upon extracts from plants, dyes, and microbiological extracts for such substances. Examples of such substances include the pain reliever aspirin, the anti-cancer drug paclitaxel (brand  
30 name Taxol<sup>TM</sup>), and the heart medication called digoxin. The number of useful medicines has generally been limited.

Purified substances having desirable bio-active properties are also often difficult to discover. Advances in traditional organic chemistry and more recently the rapid chemical synthesis methods often referred to as combinatorial chemistry have increased the number of compounds that researchers test for biological activity. Originally, substances were often initially tested on animals or humans to determine their biological activity. While results from such tests may identify a good drug candidate, they are often time consuming and costly, thus a limited number of substances can be tested. Therefore, pharmaceutical companies have turned to testing their ever-increasing libraries of substances against isolated proteins (drug targets) in biochemical assays that can be carried out at high throughput and low cost. It should be noted that the substances need to be tested in numerous protein tests, each customized for a particular drug target. Therefore, although each protein test may be run at a high-throughput, the design of multiple protein tests can be time-consuming. Substances deemed promising based on results from the protein tests are then tested in lower throughput cellular and animal tests.

There have been some attempts to use image acquisition techniques to screen a large number of substances based upon biological cell information. One such attempt is described in International Application No. WO 98/38490 in the names of Dunlay, et al. Dunlay et al. generally describes a conventional image acquisition system. This conventional system collects and saves images based on certain criteria that are predefined, not on a fixed area of an imaging surface. Additionally, the conventional system has poor lighting design, which makes image processing for multiple cells difficult. Furthermore, the conventional system is not designed for capturing, populating and utilizing a large database design. The conventional system is designed for customized cellular assays, not as a tool for generation of a cellular informatics database. Without such database capabilities the conventional system cannot be used for screening, analyzing, and comparing large quantities of cells from multiple experiments on multiple days in a predictive, efficient and cost effective manner.

What is needed is a rapid assay to assess the activity of compounds against multiple drug targets simultaneously in a cellular context. What is also needed are techniques for finding the effects of substances on cell function based upon searching and analyzing cellular information.

### SUMMARY OF THE INVENTION

According to at least one embodiment of the present invention, techniques for determining information about effects of potential substances on cells are provided. In another exemplary embodiment, the present invention provides a novel system including hardware, computer codes, user interfaces, and a database for acquiring, storing and retrieving cellular and substance information. The cells can include living, dead, or fixed cells or fractions of cells. The present invention enables, *inter alia*, researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database.

According to the present invention, a computer program for identification and verification of biological properties of substances can include code that causes a sample of a substance to be administered to a cell. The code determines one or more features for two or more cell components, or markers, in the presence of the substance. The code can form one or more descriptors from the features. Descriptors can be formed by combining features of two or more cell components as identified using the markers. The code can then search one or more descriptors obtained from prior administered substances upon cells in order to locate descriptors having a relationship to the descriptors noted for the substance under study. The code predicts properties of the administered substance based upon the properties of the prior administered substances using the relationship between the descriptors. The code can provide for identifying properties of substances based upon effects on cell characteristics. Candidate drug mechanisms of action, potency, specificity, pharmacodynamic, and pharmacokinetic parameters, toxicity, and the like can be used as substance properties.

In a specific embodiment, the present invention provides a system for acquiring knowledge from cellular information. The system has a database comprising a database management module ("DBMS"). The system also has a variety of other modules, including a population module that is coupled to the DBMS and serves to categorize and store a plurality of features (including but not limited to cell size, distance between cells, cell population, as well as sub-cellular features such as organelle location, protein location and sub-cellular constituent location and

movement) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of a descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.

In a specific embodiment, the present invention provides a system for populating a database with cellular information. The system includes a cell holder (e.g., multi-well plate, chip, microfluidic assembly, or other cell chamber) comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. Note – the light guide is one embodiment, but we don't want to be limited to it.

According to one embodiment, the present system also has an illumination apparatus including a liquid light guide operably coupled to the imaging device for highlighting the plurality of cells in a relatively even spatial manner for image capturing and measurement purposes. Still further, the liquid light guide allows sub-elements (e.g., filter, lamp) of the illumination apparatus to be placed at a remote location to prevent mechanical interference of the cell holder during image capturing. Alternative lighting methodologies may, with equal facility, be implemented.

The system also has an image-capturing device (e.g., charge coupled device camera, translation stage, shutter, microscope, software, shutter control) coupled to a computing device (e.g., computer, network computer, work station, analog computing device, on-board image-processor, and laptop). The image-capturing device is adapted to capture at least one image in at least one of the plurality of sites. One some embodiments, multiple images can be captured, where each image represents a different cell component (or portion). The image-capturing device can be adapted to convert the image into a digital representation, which highlights the feature or features of the one site.

A database storage device (e.g., relational database, object oriented database, mixed object oriented database) includes a database management element. The

database is coupled to the image capturing device. In a specific embodiment, the present system includes modules for feature extraction, generation of descriptions, and data preparation and analysis.

In a specific embodiment, the present invention provides a novel  
5 system for determining an effect of a manipulation of a cell using one or more image frames. The system has a plate comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. The system also has an image capturing device to capture a plurality of images of at least one site from the plurality of sites. The image capturing device is coupled to the computing  
10 device. The system also has an image processing device to combine the plurality of images of at least one site or plurality of sites. The image processing device is operably coupled to the plate. An image processing device is also included. The image processing device can be adapted to form a digitized representation of the plurality of images from the site or plurality of sites. Furthermore, the system has a  
15 database storage device comprising a database management element. The database can be adapted to retrieve the descriptor or descriptors of the plurality of features from the computing processing device and storing them in a selected manner.

In a specific embodiment, the present invention provides a system for capturing cellular information. The system also has an image acquisition system  
20 comprising a charged coupled device camera adapted to capture an image of a plurality of manipulated cells in various stages of the cell cycle. The stages of the cell cycle are currently understood to include interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase. The principles of the present invention specifically contemplate the application thereof on  
25 additional cell cycle stages when and if they are identified.

An optical source is coupled to the image acquisition system for highlighting the plurality of manipulated cells in the various stages of the cell cycle. The illumination apparatus provides for an acquisition of the image of the plurality of manipulated cells. In a specific embodiment, the illumination apparatus has a liquid  
30 light guide coupled to a light source at a remote location.

A variety of user interfaces are utile for accessing the several features of the present invention. Those having ordinary skill in the art will appreciate that different user interfaces may be required to support different research scenarios. The

present invention specifically contemplates the utilization of a wide variety of user interfaces.

Numerous benefits are achieved by way of the present invention over conventional techniques. The present invention can provide techniques for predictive cellular bioinformatics that can streamline a number of important decisions made in the drug discovery industry. The present invention can be implemented using off the shelf hardware including databases. In other aspects, the present invention can find useful information about substances as well as cells or portions of cells. Furthermore, the present invention can acquire more than one feature using more than one manipulation. Moreover, the present invention can provide information about a wide variety of cellular information that is not conventionally available. This information includes information about different cell components, e.g., nuclei and Golgi apparatus. Still further, the present invention provides an automated or semi-automated technique for acquiring images and populating a database. The present database can be combined with others such as genomics, and the like. Moreover, the present invention can be implemented to predict, *inter alia*, a mechanism of action, toxicity, target validation, and pre-clinical disease model.

A further understanding of the nature and advantages of the invention herein may be realized by reference to the remaining sections of the specification and the attached drawings.



### BRIEF DESCRIPTION OF THE DRAWING

For more complete understanding of the present invention, reference is  
5 made to the accompanying Drawing in the following Detailed Description of the  
Invention. In the drawing:

Fig. 1 is a simplified system diagram according to an embodiment  
according to the present invention;

10 Figs. 1A-1B are more detailed diagrams of database systems according  
to embodiments of the present invention;

Fig. 2 is a simplified block diagram according to an alternative  
embodiment according to the present invention;

Figs. 3-6 are simplified diagrams of system elements according to  
embodiments of the present invention;

15 Figs. 7A-7K illustrate representative block diagrams of simplified  
process steps in a particular embodiment according to the present invention;

Fig. 8A-8F illustrate representative quantified descriptors of effects of  
manipulations on images of cells in a particular experiment;

20 Fig. 9 illustrates example images for different types of morphologies in  
a particular experiment;

Fig. 10 illustrates a distribution of various morphologies in a cell  
population responsive to drug concentration in a particular experiment;

Fig. 11 illustrates a graph of quantified features of effects of  
manipulations on cells in a particular experiment;

25 Fig. 12 illustrates effects of external agents on cells in a particular  
experiment;

Fig. 13 illustrates 4 panels for each marker for a plurality of A549 cells  
in a particular experiment;

30 Fig. 14 illustrates 4 panels for each marker for a plurality of OVCAR-3  
cells in a particular experiment;

Fig. 15 illustrates 4 panels for each marker for a plurality of OVCAR-3  
cells at 20x in a particular experiment;

Fig. 16 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 40x in a particular experiment;

Fig. 17 illustrates a representative input for a morphometric analysis program in a particular embodiment according to the present invention; and

5 Figs. 18-19 illustrate examples of the generation of pseudo-sequences and clustering in a particular embodiment according to the present invention.

Fig. 20 is a block diagram for a first research scenario;

Fig. 21 is a block diagram for a second research scenario; and

Fig. 22 is a block diagram for a third research scenario.

10 Reference numbers refer to the same or equivalent parts of the invention throughout the several figures of the Drawing.

### DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, techniques for determining information about manipulated cells or substances based upon living, fixed, or dead cell structures or portions of cells are provided. In an exemplary embodiment, the present invention provides a novel system including computer codes coupled to a database and user interfaces for acquiring, storing and retrieving such information. Other embodiments provide a novel image capturing system for providing digitized representations of live and dead cell structures or the like.

Fig. 1 is a simplified system diagram 10 of a cellular knowledge-based system according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present system 10 includes a variety of elements such as a computing device 13, which is coupled to an image processor 15 and is coupled to a database 21. The image processor receives information from an image capturing device 17, which image processor and image capturing device are collectively referred to as the imaging system herein. The image capturing device obtains information from a plate 19, which includes a plurality of sites for cells. These cells can be biological cells that are living, fixed, dead, cell fractions, cells in a tissue, and the like. The computing device retrieves the information, which has been digitized, from the image processing device and stores such information into the database. A user interface device 11, which can be a personal computer, a work station, a network computer, a personal digital assistant, or the like, is coupled to the computing device.

Fig. 1A is a simplified diagram of a database system 1000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. Database system 1000 includes a variety of techniques for processing images from biological cells, e.g., fixed, living, and dead cells, and cell portions. As shown, images are acquired 1001. These images can be from a single frame or multiple frames. As merely an example, an image processing system may analyze such images. One example of

such an image processing system is described below, but should not be construed as limiting certain claims.

In a specific embodiment, cell samples are manipulated using a compound (e.g., substance, drug). The cell samples are imaged for a simple portion or portions, e.g., manipulated cell substructure, manipulated spatial feature of cell, cell density. Image processing techniques are used to extract 1003 the feature or features from the image or images. The features can be an independent or a dependent set of cell characteristics (which may be predominately visual) including, for example, count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface, average intensity, total intensity, optical density, radial dispersion, texture difference, and others. Each of these features corresponds to a similar manipulation by a compound. Each manipulation forms a new set of features, which are identifiable to the compound. Once each set of features has been extracted, the feature set is populated 1004 into a database 1012. Accordingly, the database includes many sets of features, where each set corresponds to a different manipulation for a selected cell. Each set of features corresponding to a manipulation provides a descriptor 1009, which is also stored 1019 in the database. The descriptor is a "finger print" including each feature for the manipulation. Each descriptor may be unique, or may have similarities to other descriptors or may even be the same as other descriptors for known and unknown manipulations.

The present system retrieves features, which we define as simple features herein, and forms composite features 1007 from them. More than one feature can be combined in a variety of different ways to form these composite features. In particular, the composite feature can be any function or combination of a simple feature and other composite features. The function can be algebraic, logical, sinusoidal, logarithmic, linear, hyperbolic, statistical, and the like. Alternatively, more than one simple feature can be combined in a functional manner (e.g., arithmetic, algebraic). As merely an example, the composite feature equals a sum of feature 1 and feature 2, where these features correspond to the same manipulation. Alternatively, the composite feature equals feature 1 divided by feature 2. Alternatively, the composite feature equals feature 1 minus feature 2. Alternatively,

the composite feature equals a constant times feature 1 plus feature 2. Of course, there are many ways that the composite feature can be defined. The present system also stores 1017 these features in the database. The composite features can also be further combined with simple features. Once these features are defined as descriptors, they are stored 1019 in the database.

Fig. 1B is a simplified diagram of a database system engine 2000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. The engine can be implemented into the present database for populating, searching, and predicting compound or cell characteristics. As merely an example, engine 2001 includes an input/output module 2008. The input/output module is used to input and output information from the database. The information includes, among others, a plurality of feature sets, which correspond to many manipulations. Additionally, the information includes descriptors, which each corresponds to a set of features from the manipulation. The database also has a population module, which is used to configure the features based upon an entity relationship, which has been predetermined.

The database engine also has other modules. In particular, the database has a transcription module, which transfers a preselected set of features and creates a descriptor from them. The transcription module can be used to take a known compound, which has features, to transcribe them into a descriptor. Alternatively, the transcription module can be used to take an unknown compound, which has features, to transcribe them into a descriptor. These descriptors are provided into the database for subsequent use. Finally, the database engine has a prediction module, which can be used to potentially predict a property (e.g., mechanism of action) of an unknown compound. Here, the unknown compound is provided with a descriptor, but the property of the compound is unknown. In one embodiment, the prediction module compares a descriptor of an unknown compound with the many descriptors of known compounds, which were in the populated database. Depending upon the matching criteria, the prediction module will attempt to uncover one or more descriptors of known compounds. Once the prediction module finds the descriptors of the known compounds based upon the descriptor for the unknown compound, it identifies a potential property of such unknown compound for analysis and review. Here, it is

believed that certain features of the known compound, which are similar to those features of the unknown compound may uncover a property to the unknown compound. Details of the present software engine are described more fully below.

Fig. 2 is a simplified block diagram 20 of a cellular knowledge-based system according to an alternative embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Like reference numerals are used in the present diagram as the previous diagram for easy cross-referencing, but are not intended to be limiting in any manner. The present diagram 20 includes a variety of elements such as a processor 13 or computing device coupled to a database 11. The processor can be used for retrieving and storing information from the database. The system also includes a plurality of system elements, such as a cleaner 23, a dispenser 25, and an image capturing system 27, which are also coupled to the database in some embodiments. These elements can be coupled to each other through a network or the like. As merely an example, the network can be a NetWare<sup>TM</sup> network from Novell Corporation or an internet network or the Internet but can also be others and any combination thereof. The system also has an output device 31, which can be used to output information from the database, processor, or other system elements. Details of these elements are described more fully below in reference to the Figs.

Figs. 3-5 are simplified drawings of system elements according to embodiments of the present invention. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. As merely an example, Fig. 3 is a simplified diagram of a processor or computing device 13. The computing device 13 includes a bus 112 which interconnects major subsystems such as a central processor 114, a system memory 116 (e.g., random access memory), an input/output ("I/O") controller 118, an external device such as a display screen 124 via a display adapter 126, a keyboard 132 and a mouse 146 via an I/O controller 118, a SCSI host adapter (not shown), and a floppy disk drive 136 operative to receive a floppy disk 138.

The computing device has other features. Storage Interface 134 may act as a storage interface to a fixed disk drive 144 or a CD-ROM player 140 operative

to receive a CD-ROM 142. Fixed disk 144 may be a part of computing device or may be separate and accessed through other interface systems. A network interface 148 may provide a direct connection to a remote server via a telephone link or to the Internet. Network interface 148 may also connect to a local area network ("LAN") or other network interconnecting many computer systems. Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 3 to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 3. The operation of a computer system such as that shown in Fig. 3 is readily known in the art and is not discussed in detail in this application. Computer code to implement the present invention, may be operably disposed or stored in computer-readable storage media such as system memory 116, fixed disk 144, CD-ROM 140, or floppy disk 138. The computer code can be organized in terms of processes or modules, depending upon the application. That is, the computer code can include a prediction module, a translation module, or other modules to carryout the functionality described herein, as well as others.

Figs. 4 and 5 are simplified diagrams of an imaging system 200 according to an embodiment of the present invention. As shown, the imaging system 200 includes a variety of features such as housing 203, which holds a stage assembly 204. The stage assembly includes an x-stage movement element 206, which is along an x-direction, and a y-stage movement element 207, which is along a y-direction. The imaging system also includes a z-direction movement element, which is perpendicular to the x-y plane. The z-direction movement motor can be attached to the stage, or to the objective nosepiece by way of the microscope housing, or as an external motor between the objective and the microscope housing. The stage can align in any one of the directions to an accuracy of one micron and less, or one-half micron and less, or one-quarter micron and less, depending upon the embodiment.

The stage holds a plate 202 or cell holder, which houses one of a plurality of samples. The plate includes a spatial array 209 of process sites. Each of the process sites can include a plurality of cells and solutions depending upon the embodiment. Each of the sites can carry a sufficient amount of solution to prevent substantial evaporation of the sample during processing in some embodiments. In embodiments for large scale analysis, the plate includes at least 96 sites, or more than

or equal to 384 sites, or more than or equal to 1,536 sites. The plate bottom is transparent and thin, which allows light to pass through the sample. Additionally, the plate is made of a suitable chemical resistant material. As merely an example, the plate can be either a 96, or 384, or 1536 or other formats from places such as Becton Dickinson of Franklin Lakes, NJ, or Corning Science Products of Corning, NY. In a preferred embodiment, the plate is a Corning Costar black-walled 96 well plate catalog #3904 from Corning Science Products of Corning, NY, but should not be limited to these in some applications, but can be others.

Also shown is the condenser for the microscope 201, which can be used to collect phase, DIC, or bright field images of the cells. Images resulting from the illumination of the samples to fluorescence, phase, DIC, or bright field techniques are collected using an image capturing device 208, which captures an image or images of cells from the plate. In a specific embodiment, the microscope is an inverted configuration with the objectives on the bottom of the plate and the condenser disposed overlying an upper surface of the sites, while the image capturing device underlies the sites. Images captured by the imaging device, whether analogue or digital, are viewed by a monitor or other devices. The image capturing device can be any camera assembly such as a charge coupled device camera, which is known as a CCD camera, or other high resolution camera capable of capturing images from the sites. In a specific embodiment, the camera is an interline CCD camera which does not require an external shutter.

In a specific embodiment, the present imaging system can be any suitable unit that is flexible for automated image collection using multi-well plastic plates. The imaging system also should be adapted to collect high-resolution images of cells on plastic or glass plates, cell growth chambers, or coverslips. The system also can be used for imaging multiple cell markers in multiple imaging conditions. To accomplish this, the microscope system has a variety of elements such as a light source, a motorized excitation filter wheel and shutter, x-y-z-motorized stage, excitation and emission filters, Fluor phase and DIC objectives, motorized objective nosepiece, dichroic filters, motorized dichroic filter cubes, phase and DIC rings and prisms, CCD camera, and software control. As merely an example, the present imaging system can have components such as those listed in the Table below.



DESCRIPTION	MAKER	MODEL
Microscope	Zeiss	100M
(x-y) motorized stage	Prior	
Xenon lamp	Sutter	Lambda
Filter wheel	Sutter	Lambda-10
Microtitre Plate holder	Prior	500-H223R
Isolation Table	Kinetic Systems	9101-24-85
Objective Spacers	Polytec PI	P-721.90
Camera	Hamamatsu	C47-95
Computer	IBM	IntelliStation
Software	Metamorph	v.4
Objectives	Zeiss	Achroplan 10x/0.25 LD-Achroplan 20x/0.4 LD-Achroplan 40x/0.6

Table: Image Acquisition System Elements

5 In a specific embodiment, the present system has the following capabilities, which are not intended to be limiting.

Image acquisition

- 1) Ability to automatically acquire multi-wavelength images from multiple sites on one multi-well plate, to sequentially name image files, and to log any  
 10 imaging parameter information with image files.
- 2) Ability to link images with a larger database/spreadsheet of information.
- 3) Ability to automatically collect multiple plates by interfacing the imaging system with a robotic arm.

15

X-Y control

- 1) Ability to place 96, 384, or 1536 well plates onto microscope stage and move to each well sequentially.

2) Ability to return to each well and collect another round of images (multi-site time-lapse) or ability to collect rapid time-lapse information at each well (time-lapse of many wells).

3) Ability to collect a low magnification image, automatically determine features which may be of interest, automatically change the objective to a higher magnification, and collect high magnification images of a fixed number of those identified cells in the sample.

4) Ability to collect multiple frames in each site.

10 Z control

1. Ability to auto-focus with substantially minimal damage to biological specimen or fluorophore.

2. Ability to auto-focus rapidly.

15 The present embodiment of the imaging system is shown by way of Figs. 5A and 5B. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present imaging system 40 includes a variety of elements such as a microscope 41, which is preferably an epi-fluorescent microscope, but can be confocal, multiphoton, or hybrid types. The microscope includes elements  
20 41A, the motorized Z-axis; 41B, the motorized dichroic filter cube holder; and 41C, the motorized objective nosepiece. In one embodiment, the microscope is a Model 100M made by Zeiss. The microscope communicates to computer 51 through control lines 73, 75, and 76. The imaging system also has camera 50 coupled to controller  
25 50A and computing device 51, which oversees and controls operations of the elements of the imaging system.

The present microscope includes drivers for spatially moving a stage in two dimensions, including an x-direction, a y-direction, and moving the objective nosepiece in a z-direction in a Cartesian coordinate system. The z-direction  
30 movement is provided using a fast z-motor, which can make z-direction adjustments within a predetermined time. The z-direction movement generally provides for focussing of the sample to the camera. The focussing occurs within the predetermined time of preferably ten seconds and less, or five seconds and less, or one

second and less, depending upon the embodiment. As merely an example, the z-motor or positioner can be a model PIFOC objective nanopositioner made by a company called Physik Instrumente of Waldbronn, Germany, but also can be others. The z-motor couples to computer 51 through line 63, which may also include a  
5 controller. Depending upon the embodiment, a second z-motor 41A connected to the computer 51 by line 73 may be used to keep the z-motor 42 in the center of its travel. Alternatively, in other embodiments the stage could be provided with a z-motor allowing for movement of the stage in the z-direction.

The present stage also includes an x-y stage 43. The x-y stage moves  
10 plate 59, e.g., 96 site, 384 site, 1536 site. The x-y stage moves plate in an x-y spatial manner. The stage has an accuracy or repeatability of about 1 micron and less, or about 2 microns and less. The stage can move in a continuous manner or a stepped manner. The stage also can move up to 30 mm/sec. or faster. The stage also can move 1 mm/sec. and less, depending upon the embodiment. The stage can also step  
15 0.1 micron and less or 1 micron and less, as well as other spatial dimensions. The stage can be one such as a Proscan Series made by Prior Scientific of Rockland, MA but can also be others. The stage is controlled via control line 61 through controller 43A, which couples to computer 51 through control line 65.

The stage includes plate holder 44. The plate holder can hold a single  
20 plate. In other embodiments, plate holder can also hold multiple plates. The plate holder can use mechanical, electrical, fluid, vacuum and other means for holding the plate or plates. The plate holder also is sufficiently stable for securing the plate. As merely an example, the plate holder is a Model 500-H223R made by Prior Scientific of Rockland, MA. In some embodiments, the plate holder may need adjustment in  
25 the z-direction to provide for a desirable focus of a sample on a plate. In these embodiments, the plate holder is supported by spacers 45 or a plurality of stage pins, which mechanically elevate the plate holder in the z-direction. These pins are generally made of a suitable material for supporting such plate holder and also are sufficiently resistant to chemicals and the like.

30 In some embodiments, the entire imaging system is placed on an isolation table 49. The isolation table is disposed between the microscope and support structure. The isolation table is designed to prevent excessive vibration of the plate. The isolation table is made of a suitable material such as steel and honeycomb but can

be others. The table has a thickness of about 8 inches or preferably less than about 24 inches. In one embodiment, the table is Model 9101-24-85 made by Kinetic Systems of Boston, MA.

The imaging system also has a lamp or illumination assembly 62. The lamp assembly provides for a light source (See reference letter B) to a plurality of elements in the imaging system. For easy reading, the light path is defined by the dotted lines, which are not intended to be limiting. The lamp assembly has a variety of elements such as a Xenon lamp 46. The Xenon lamp provides light at about 320 to 700 nanometers (Prefocused). The Xenon lamp is 175 or 300 Watts. As merely an example, the lamp can be a Lambda Model made by Sutter Instrument Company of Novato, CA.

Referring to Fig. 5B, the lamp assembly also has a cold mirror 58, an excitation filter wheel 48, excitation filter(s) 55, and an excitation light shutter 57. As shown, light is derived from the Xenon lamp, reflects off of the cold mirror 58, traverses through the excitation filter or filters 55, and is controlled by the excitation light shutter 57. The lamp assembly has filter wheel 48, which houses one of a plurality of filters, including excitation filters. The shutter and filter wheel are controlled via control lines 67, which are coupled to a computer 51 or other type of computing device. The control lines 67 are coupled through controller 57A (for element 57) and controller 48A (for element 48) via control line 69 to computer 51.

Preferably, light traverses from the lamp assembly through a light guide 47 to illuminate features within the plate. The light guide is suitably selected to have a flexible member, which can be used to place lamp source at a remote location away from the imaging device. The flexible member substantially keeps any vibration from the lamp assembly away from the imaging device. In some embodiments, the member is at least 1 foot away from the imaging device. The light guide is a guide, which is a flexible hose-type sleeve. The sleeve is filled with a liquid such as an aqueous solution containing chloride or phosphate. A thin layer may be formed on the inside of the sleeve. The layer can be a containing tetrafluoroethylene and hexafluoropropylene, or containing tetrafluoroethylene and perfluoromethyl vinyl ether, or tetrafluoroethylene and perfluoropropyl vinyl ether. An example of such a light guide is described in International Application No. WO/98/38537 filed February 29, 1997, and assigned to NATH, Gunther. The liquid

light guide has less than about 30% transmission loss of the light at a remote location such as the imaging system.

Light is derived from the lamp assembly and directs off of filter 56, which directs the light upward. Filter 56 can be a dichroic and emission filter, as well as others. The light traverses through microscope nosepiece 41C, and traverses through objective spacers 54. An objective 53 magnifies the light toward a predetermined point on the plate 59. The objective can be, for example, made by Zeiss of Jena, Germany, as well as other companies. The objective can be one of a plurality including 1X, 10X, 20X, 40X, and others, depending upon the application. Magnification can be further expanded or contracted by intermediate optics between the objective and the camera. Selection of filter or filters is controlled by computer 51 via control line 75.

The camera 50 captures an image of cells from plate 59. The image is obtained from light scattering off of cells or portions of cells in the plate through objective 53, through objective spacers, through filters 56, which are captured at camera 50. In this preferred embodiment, the camera is a digital camera, but can be an analogue camera. The digital camera is a CCD camera, which has 1280 by 1024 pixels, or more or less. The pixels can be 6.7 microns in dimension or more or less. The camera preferably is substantially free from an external shutter to quickly capture a plurality of images of cells from the plate. The camera is controlled via control line 71 through controller 50A, which connects to computer 51 through control line 70. The present invention can also include other types of image acquisition devices selected from at least an epifluorescence, a confocal, a total-internal reflection, a phase, a Hoffman, a bright field, a dark field, a differential interference contrast, an interference reflection, or multi-photon illumination device.

The present imaging system stores images on a high density memory device 60. The high density memory device is preferably optical, but can also be magnetic. The high density memory device can be any suitable unit that is capable of storing a plurality of images from a plurality of sites in the plate. The memory device can be a compact disk, which would generally use a compact disk burner or the like. Depending upon the embodiment, the high density memory device is used to archive the images that are captured from the camera in the imaging system. Further details

of the imaging system can be found throughout the present specification, and more particularly below.

As merely an example, the present invention can be implemented using the following sequence of steps, which have been described in a journal entry form.

- 5 Here, images are opened and objects are identified based on a background value that has been edited in starting image acquisition. Information is maintained in a spreadsheet or other database format, which has the following information for each object:

Image Name	Image Plane	Image Date and Time
Elapsed Time	Object #	Total area
Pixel area	Area	Hole area
Relative hole area	Standard area count	Perimeter
Length	Breadth	Fiber length
Fiber breadth	Shape factor	Ell. form factor
Inner radius	Outer radius	Mean radius
Average gray value	Total gray value	Optical density
Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis
EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major Axis Angle	EFA Harmonic 2, Ellipse Area
EFA Harmonic 2, Axial Ratio	EFA Harmonic 3, Semi-Minor Axis	

10

After computations are done, the log file is saved. In particular, the file is saved in an appropriate place with an appropriate name.

In a specific embodiment, the present invention provides the following detailed example of journal entries, which should not limit the scope of the invention.

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)
Stage (Log Position)	
Stage (Scan Wells)	User picks wells to scan: runs 3x3 image collection.jnl.

3X3 IMAGE COLLECTION.jnl

Stage (Scan)	Takes 9 images of well, -1600 motor steps apart from left to right 3 columns and 3 rows, runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL.
--------------	--

5

FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl.

Stage (Log Position)	Logs stage position of each image
ADC – Focus	Opens up the manual focusing window with whatever focus time is current set
Show Message and Wait	Interactive: user hits enter to continue when done focusing

ADC-Acquire from Digital Camera	Takes Hoechst image
Save Using Sequential File Names	
Close	Closes image window

## START IMAGE ANALYSIS.jnl

Low Pass	3x3 convolution of already opened image
Low Pass	3x3
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 4. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 3. into the constant Value field
Threshold image	Creates threshold 1 unit above 0 to 4096
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 8.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 7. into Standard Area box to be used by automated IMA for all images



IMA OBJECTS.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS.jnl step 3
Threshold Image	1 unit above 0
Integrated Morphometry – Load State	Hoecsht.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

Log obj and sum data.jnl

Integrated Morphometry – Log Data	Logs object data into Sheet 1
Integrated Morphometry – Log Data	Log summary data into Sheet 2

5

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET.jnl

Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Loops IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save Excel spreadsheet

OPEN OBJECT LOG DDE FILE.jnl

Open Object Log	Opens a DDE object log into sheet 1 of an Excel spreadsheet
Open Summary Log	Opens a summary log into sheet 2

## COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET 16 BIT IMAGES.jnl

Arithmetic	Interactive: Opens Arithmetic window for user to input background subtraction level from START IMAGE ANALYSIS.jnl step 3
Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Interactive: Runs IMA OBJECTS 16 bit.jnl. User picks directory from which to choose.

5

IMA OBJECTS 16bit.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Copy to 8-bit Image	No autoscale, to new untitled image
Save Using Sequential File Name	Saves 8bit image using previously defined Sequential File names.
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS16 TO 8 BIT.jnl step 5
Threshold Image	1 unit above 0 to 255

Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

## START IMAGE ANALYSIS 16 to 8 BIT.jnl

Copy to 8-bit Image	No autoscale, to new untitled image
Close	Closes 16 bit image
Low Pass	3x3 convolution
Low Pass	3x3 convolution
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 6. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 5. into the constant Value field
Threshold image	Creates threshold by 1 unit above 0 to 255
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 10.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 9. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS WITH NEW LOG FILE.jnl

Run Journal	OPEN OBJECT LOG DDE FILE.JNL
Run Journal	IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save every Excel spreadsheet generated.

INTERACTIVE IMA OBJECTS.jnl

Threshold Image	User manually sets threshold
Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Objects
Integrated Morphometry – Log Data	Into open object.log file

5

COLLECT INTERACTIVE IMA DATA.jnl

Close Object Lo g	
Open Object Log	Interactive
Annotate Log File	Interactive: experimental information that will go into the first line of the object log file
Loop for all Images in Directory	Runs INTERACTIVE IMA OBJECTS.jnl

CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE  
NAME.jnl

Stage (Log Position)	
ADC-Focus	

Show Message and Wait	Interactive – user presses Enter when done focusing
ADC – Acquire from Digital Camera	Hoechst
Save Using Sequential File Name	
Close	Close open image

COLLECT HOECHST AND FITC.jnl

Run Journal	FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL
Run Journal	CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

3X3 IMAGE COLLECTION HOECHST FITC.jnl

Stage (Scan)	COLLECT HOECHST AND FITC.jnl
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5

AUTOMATED 3X3 IMAGE COLLECTION HOECHST FITC.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Excel DDL files
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)

Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs 3X3 IMAGE COLLECTION HOECHST FITC.jnl

AUTOMATED IMAGE COLLECTION.jnl

Sct Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL. Well to well travel = (-9035, -9035)

5

STARTUP.jnl

Install and Configure Devices	Open Stage Meta Devices
Set Live Video Channel	

Preferences	<u>Measure Objects</u> : Draw failed classifier objects, Exclude objects that touch the edge of the image, Enable Elliptical Fourier Parameters, <b>turn off</b> Warn users when measurement data will be erased <u>Image Saving</u> : Save Tiff/stk using LZW compression <u>Image Windows</u> : Use transparent thresholds.
Configure Default Paths	C:\Metamorph Data C:\Metamorph Data\Common Settings
Load Journal Taskbar	Common.JTB

### Nested Journals

#### Automated 3x3 Image Collection

5

*Loop 3x3 image collection*

*Loop focus, collect image, save sequential file name*

#### Automated 3x3 image collection Hoechst FITC

10

*Loop 3x3 image collection Hoechst FITC*

*loop Collect Hoechst and FITC*

*focus, collect image, save sequential file name*

*change filter, collect image, save sequential file name*

#### Automated image collection

15

*Loop focus, collect image, save sequential file name*

#### Collect automated IMA data in one Spreadsheet

*Open object log DDE file*

*Loop* IMA objects

Log obj and sum data

Collect automated IMA data in one spreadsheet 16 bit images

5           Open object log DDE file

*Loop* IMA objects 16 bit

Log obj and sum data

10           Although the above has been generally described in terms of a specific user interface and software code, other user interfaces and code can also be used. One of ordinary skill in the art would recognize many other variations, alternatives, and modifications.

Fig. 6 is a simplified diagram 600 of a cleaning and dispensing system according to an embodiment of the present invention. This system 600 includes a  
15   variety of elements such as a dispensing head 609, which is coupled to a plurality of pipettes 601. The pipettes input and output fluids or solutions from plate 603. The plate has a plurality of sites, each of which can be used to input cells or a combination of cells and solution. The system also has elements to house solutions 605, which are used to manipulate cell samples in the plate. The dispensing head is supported  
20   through a support member 607, which is sufficiently rigid to allow for movement of the head. The dispenser is coupled to the present system in a mechanical and electrical manner, which provides for a fully integrated system for providing cell samples to the imaging system according to the present invention.

Fig. 7A illustrates a representative block flow diagram of simplified  
25   process steps of a method for determining properties of a manipulation based upon effects of the manipulation on one or more portions of one or more cells in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. In step  
30   700, one or more samples of cells can be provided. These cells can be live, dead, or fixed cells, or cell fractions. The cells also can be in one of many cell cycle stages, including G0, G1, S, G2 or M phase, M phase including the following cell cycle stages: interphase, prophase, prometaphase, metaphase, anaphase, and telophase.



Cell components tracked in presently preferable embodiments can include proteins, protein modifications, genetically manipulated proteins, exogenous proteins, enzymatic activities, nucleic acids, lipids, carbohydrates, organic and inorganic ion concentrations, sub-cellular structures, organelles, plasma membrane, adhesion complex, ion channels, ion pumps, integral membrane proteins, cell surface receptors, G-protein coupled receptors, tyrosine kinase receptors, nuclear membrane receptors, ECM binding complexes, endocytotic machinery, exocytotic machinery, lysosomes, peroxisomes, vacuoles, mitochondria, Golgi apparatus, cytoskeletal filament network, endoplasmic reticulum, nuclear membrane, proteosome apparatus, chromatin, nucleolus, cytoplasm, cytoplasmic signaling apparatus, microbe specializations and plant specializations.

The following table illustrates some markers and cell components commonly used by embodiments according to the present invention. Other markers can be used in various embodiments without departing from the scope of the invention.

Cell component	Marker	Disease State
Plasma membrane (including overall cell shape)	Carbocyanine dyes Phosphatidylserine Various lipids Glycoproteins	Apoptosis-Cancer Apoptosis-Neural degenerative Ds
Adhesion complexes	Cadherins Integrins Occludin Gap junction ERM proteins CAMs Catenins Desmosomes	Thrombosis Metastasis Wound healing Inflammatory Ds Dermatologic Ds
Ion Channels and Pumps	Na/K Atpase Calcium channels Serotonin reuptake pump CFTR	Cystic fibrosis Depression Congestive Heart Failure Epilepsy

G coupled receptors	$\beta$ adrenergic receptor Angiotensin receptor	Hypertension Heart Failure Angina
Tyrosine kinase receptors	PDGF receptor FGF receptor IGF receptor	Cancer Wound healing Angiogenesis Cerebrovascular Ds
ECM binding complexes	Dystroglycan Syndecan	Muscular Dystrophy
Endocytotic machinery	Clathrin Adaptor proteins COPs Presenilins Dynamin	Alzheimer's Ds
Exocytotic machinery	SNAREs Vesicles	Epilepsy Tetanus Systemic Inflammation Allergic Reactions
Lysosomes	Acid phosphatase Transferrin	Viral diseases
Peroxisomes/Vacuoles		Neural degenerative Ds
Mitochondria	Caspases Apoptosis inducing factor F1 ATPase Fluorescein Cyclo-oxygenase	Apoptosis Neural degenerative Ds Mitochondrial Cytopathies Inflammatory Ds
Golgi Apparatus	Lens Culinaris DiOC6 carbocyanine dye COPs	

Cytoskeletal Filament Networks	Microtubules Actin Intermediate Filaments Kinesin, dynein, myosin Microtubule associated proteins Actin binding proteins Rac/Rho Keratins	Cancer Neural degenerative Ds Inflammatory Ds Cardiovascular Ds Skin Ds
Endoplasmic Reticulum	SNARE PDI Ribosomes	Neural degenerative Ds
Nuclear Membrane	Lamins Nuclear Pore Complex	Cancer
Proteosome Apparatus	Ubiquityl transferases	Cancer
Chromatin	DNA Histone proteins Histone deacetylases Telomerases	Cancer Aging
Nucleolus	Phase markers	
Cytoplasm	Intermediary Metabolic Enzymes BRCA1	Cancer
Cytoplasmic Signaling Apparatus	Calcium Camp PKC pH	Cardiovascular Ds Migraine Apoptosis Cancer
Microbe Specializations	Flagella Cilia Cell Wall components: Chitin synthase	Infectious Ds

Plant specializations	Choloroplast Cell Wall components	Crop Protection
-----------------------	--------------------------------------	-----------------

Then, in a step 702, one or more samples of the manipulation can be provided to the cells. Manipulations can comprise one or any combination of chemical, biological, mechanical, thermal, electromagnetic, gravitational, nuclear, or temporal factors, for example. For example, manipulations could include exposure to chemical compounds, including compounds of known biological activity such as therapeutics or drugs, or also compounds of unknown biological activity. Or exposure to biologics that may or may not be used as drugs such as hormones, growth factors, antibodies, or extracellular matrix components. Or exposure to biologics such as infective materials such as viruses that may be naturally occurring viruses or viruses engineered to express exogenous genes at various levels. Bioengineered viruses are one example of manipulations via gene transfer. Other means of gene transfer are well known in the art and include but are not limited to electroporation, calcium phosphate precipitation, and lipid-based transfection. Manipulations could also include delivery of antisense polynucleotides by similar means as gene transfection. Other genetic manipulations include gene knock-outs or gene mutations. Manipulations also could include cell fusion. Physical manipulations could include exposing cells to shear stress under different rates of fluid flow, exposure of cells to different temperatures, exposure of cells to vacuum or positive pressure, or exposure of cells to sonication. Manipulations could also include applying centrifugal force. Manipulations could also include changes in gravitational force, including sub-gravitation (the preferred embodiment in outer space). Manipulations could include application of a constant or pulsed electrical current. Manipulations could also include irradiation. Manipulations could also include photobleaching which in some embodiments may include prior addition of a substance that would specifically mark areas to be photobleached by subsequent light exposure. In addition, these types of manipulations may be varied as to time of exposure, or cells could be subjected to multiple manipulations in various combinations and orders of addition. Of course, the type of manipulation used depends upon the application.

Then, in a step 704, one or more descriptors of a state in the portions of the cells in the presence of the manipulation can be determined using the images

collected on the imaging system. Descriptors can comprise scalar or vector values, representing quantities such as area, perimeter, dimensions, intensity, gray level, aspect ratios, and the like. Other types of descriptors include, but are not limited to, one or any combination of characteristics such as a cell count, an area, a perimeter, a  
5 length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average intensity, a total intensity, and an optical density. These descriptors can be average or standard deviation values, or frequency  
10 statistics from the descriptors collected across a population of cells. These descriptors can be further reduced using other methods such as principal component analysis and the like. In some embodiments, the descriptors include features from different cell portions or cell types. That is, a first feature can be from a nuclei and a second feature is from another cell structure such as Golgi apparatus, mitochondria, spacing between  
15 cell structures or cells themselves, as well as many others.

A presently preferable embodiment uses descriptors selected from the following table. Other descriptors can also be used without departing from the scope of the invention.

Name of Parameter	Explanation/Comments
Count	Number of objects
Area	
Perimeter	
Length	X axis
Width	Y axis
Shape Factor	Measure of roundness of an object
Height	Z axis
Radius	
Distribution of Brightness	
Radius of Dispersion	Measure of how dispersed the marker is from its centroid
Centroid location	x-y position of center of mass
Number of holes in closed objects	Derivatives of this measurement might include, for

	example, Euler number (= number of objects - number of holes)
Elliptical Fourier Analysis (EFA)	Multiple frequencies that describe the shape of a closed object
Wavelet Analysis	As in EFA, but using wavelet transform
Interobject Orientation	Polar Coordinate analysis of relative location
Distribution Interobject Distances	Including statistical characteristics
Spectral Output	Measures the wavelength spectrum of the reporter dye. Includes FRET
Optical density	Absorbance of light
Phase density	Phase shifting of light
Reflection interference	Measure of the distance of the cell membrane from the surface of the substrate
1,2 and 3 dimensional Fourier Analysis	Spatial frequency analysis of non closed objects
1,2 and 3 dimensional Wavelet Analysis	Spatial frequency analysis of non closed objects
Eccentricity	The eccentricity of the ellipse that has the same second moments as the region. A measure of object elongation.
Long axis/Short Axis Length	Another measure of object elongation.
Convex perimeter	Perimeter of the smallest convex polygon surrounding an object
Convex area	Area of the smallest convex polygon surrounding an object
Solidity	Ratio of polygon bounding box area to object area.
Extent	proportion of pixels in the bounding box that are also in the region
Granularity	
Pattern matching	Significance of similarity to reference pattern
Volume measurements	As above, but adding a z axis

Then, in a step 705, a database of cell information can be provided.

Next, in a step 706, a plurality of descriptors can be searched from a database of cell information in order to locate descriptors based upon one of the descriptors of the manipulation. Then, in a step 708, properties of the manipulation are predicted based upon the properties of the located descriptors. Properties can comprise toxicity, specificity against a subset of tumors, mechanisms of chemical activity, mechanisms of biological activity, structure, adverse biological effects, biological pathways, clinical effects, cellular availability, pharmacological availability, pharmacodynamic properties, clinical uses and indications, pharmacological properties, such as absorption, excretion, distribution, metabolism and the like.

In a particular embodiment, step 706 comprises determining matching descriptors in the database corresponding to a prior administration of the manipulation to the descriptors of the present administration of the manipulation. In a particular embodiment according to the present invention, combinations of measurements of scalar values can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell-substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be analyzed, classified, and compared using a plurality of techniques, such as statistical classification and clustering, heuristic classification techniques, a technique of creating "phylogenetic trees" based on various distance measures between descriptors from various drugs. In this embodiment, numeric values for the descriptors can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound descriptor with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphological properties of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, comparisons can be performed on acquired image

features. Some embodiments can comprise statistical and neural network - based approaches to perform comparisons of various features. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data.

5                   In some embodiments, classification, clustering and other types of predictive data analysis can be performed on features extracted from cell images. In a presently preferable embodiment, statistical procedures for comparisons, classification and clustering are performed on data obtained from imaging cells.

Fragments of data preparation and pre-formatting (S language):

```
10       >tmp.frame <- Generic.Summary  
      >names1 <- paste("Cell.line.5", tmp.names, sep=".")  
      > by.compound.matrix <- as.matrix(arranged.by.compound)
```

Example of the code for principal component analysis (data  
15   preparation) using S language:

```
      all.data.princomp <- menuPrincomp(data =  
      by.compound.matrix, scores = T, cor = "Correlation",  
      na.action = T, print.short = T, print.importance = T,  
      print.loadings = T, cutoff.loadings = 0.1,  
20   plot.screplot = T, plot.loadings = T, plot.biplot = T,  
      plot.biplot.choices = c(1,2), predict.p = F)
```

Example of clustering using a divisive hierarchical clustering  
algorithm:

```
25       > div.hier.2.manhattan.cluster$call  
      diana(x = tmp.sum.by.comp, diss = F, metric =  
      "manhattan",  
          stand = T, save.x = T, save.diss = T)
```

30                   Another embodiment utilizes existing tools for biological sequence similarity searches, classification, and phylogenetic analysis. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes according to a one of several sets of rules. Once



converted into a corresponding nucleotide or amino acid sequence representation, the fingerprints can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. Select  
5 embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the PHYLIP (PHYlogeny Interference Package) a package of programs for inferring phylogenies (evolutionary trees) described in (Feldenstein, J.  
10 1996 Methods Enzymol 266:418-427 and Feldenstein, J. 1981 J. Mol. Evol. 17(6):368-376).

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. Further details of a step of manipulation are noted more particular below.

15 Fig. 7B illustrates a representative block flow diagram of simplified process steps for determining one or more descriptors of a state in the portions of the cells in the presence of the manipulation of step 704 of Fig. 7A in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill  
20 in the art would recognize other variations, modifications, and alternatives. In a step 712, an image of a cell portion is obtained. In some embodiments, the cell portion is visualized with a fluorescently labeled marker that is specific for the portion or portions of interest. A cell portion can include, for example, one or more of the following: nuclei, Golgi apparatus, and other features. The cell portion may vary in  
25 select embodiments according to the invention. Then, in a step 714, a digitized representation of the image obtained in step 712 is determined. In some embodiments, steps 714 and step 712 can comprise a single step. These embodiments use a digital imaging means such as a digital camera, to obtain a digital image of the target directly. Next, in a step 716, the digital representation of the image is  
30 processed to obtain image features. Image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Then, in a step 718 descriptors can be determined from the image features. Descriptors can comprise scalar or vector quantities and can comprise the image features themselves, as well as

composed features, such as shape factor derived by a relationship  $4\pi * \text{area} / \text{perimeter}$ , and the like. Descriptors can also comprise statistical quantities relating to feature characteristics across a population of cells, such as a standard deviation, and average, and the like.

5 In a preferred embodiment, cells can be placed onto a microscope, such as a Zeiss microscope, or its equivalent as known in the art. A starting point, named Site A01, is identified to the microscope. A plurality of exposure parameters can be optimized for automated image collection and analysis. The microscope can automatically move to a new well, automatically focus, collect one or more images, at  
10 one or more wavelengths, move to a next well, and repeat this process for all designated wells in a multiple well plate and for multiple plates. A file having a size and an intensity distribution measurement for each color and rank for each well can then be created for the images acquired. Based on this information, a user or a computer can revisit sites of interest to collect more data, if desired, or to verify  
15 automated analysis. In a presently preferred embodiment, image automatic focus and acquisition can be done using computer software controlling the internal Z-motor of the microscope. Images are taken using a 10x, 20x, or 40x air long working distance objectives. Sometimes multiple images are collected per well. Image exposure times can be optimized for each fluorescent marker and cell line. The same exposure time  
20 can be used for each cell line and fluorescent marker to acquire data.

Fig. 7C illustrates a representative block flow diagram of simplified process steps for obtaining images of cell portions of step 712 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill  
25 in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 720, a sample is provided to the imaging device. Samples can be provided in 96 well plates and the like. The sample may be loaded into a microscope, such as a Zeiss microscope or equivalent.

30 (2). In a step 722, a set of optical filters is selected to shine light of the appropriate wavelength to illuminate the first sample, which may be contained in a first well designated A01.

(3). In a step 724, an automatic focusing procedure is performed for the site. In a particular embodiment, the internal z-motor of the microscope which is attached to the objective nosepiece is used for automatic focusing of the microscope. In an alternative embodiments, the plate holding the samples is moved to perform  
5 automatic focusing of the microscope, or focusing can be performed by moving optical components attached to the microscope and the like.

(4). In a step 726, images are collected for the site. Images can be collected for every color at every site. Present embodiments can provide images for up to four colors. However, embodiments are contemplated that can provide more  
10 colors by using either a monochromator coupled with excitation filters which are on a filter wheel, or by digitally separating overlapping fluorophores. Those knowledgeable in the field will know that given calibration images of single fluorophores, a look-up table can be devised which will allow for the digital removal of fluorescence bleed-through of fluorescence which may occur in optical channels  
15 other than the one for which that filter has been optimized in instances of using more than one fluorophore at once. Cell growth and density information is also collected. Cell density is determined by what percentage of the area being imaged is inhabited by cells. In some embodiments, imaging can be facilitated using one or more biosensors, molecules such as non-proteins, i.e., lipids and the like, that are  
20 luminescently tagged. However, some embodiments can also use fluorescence polarization and the like. Fluorescence polarization is a homogeneous fluorescence technology where the excited state of the molecule lasts much longer than in normal fluorescence, taking seconds to minutes to reach equilibrium, obliterating the need to wash away fluorescence markers that are not specifically bound to a marker. Further,  
25 embodiments can detect differences in spectral shifts of luminescent markers. Some fluorescence markers, such as Nile Red sold by Molecular Probes of Eugene, OR, will change its emission peak wavelength depending on its environment. One can detect these changes by monitoring the level of fluorescence at both wavelengths and reading out at ratio of the two.

(5). In a step 728, a determination is made whether more fields of view  
30 need to be taken for a particular color. If this is so, then processing continues at step 726 at a new site. Otherwise, processing continues with a decisional step 730.

Images can now be taken by repeating step 726. In a preferred embodiment 4 to 9 images are collected at each site.

(5). In a step 730, a determination is made whether more optical configurations need to be taken in order to obtain images for all differently-marked cell portions the sample. If this is so, then in a step 732 a new optical configuration is determined. Images for the new optical configuration can now be taken by repeating steps 726 and 728.

(6). In a decisional step 734, after all optical configurations and images for fields of view in a sample have been obtained, a determination is made whether any further samples remain to be analyzed. If so, a new sample is brought into view and processing continues with step 720. Otherwise, image processing is complete. In a presently preferable embodiment, image data can be stored on a CD ROM using a CD ROM burner, such as CRW4416 made by Yamaha of Japan. However, other mass storage media can also be used.

Fig. 7D illustrates a representative block flow diagram of simplified process steps for processing digitized representations of step 716 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 740, a digitized image input is preprocessed. Preprocessing might include, but is not limited to, such operations as background subtraction, thresholding, smoothing, adoptive filtering, edge enhancements, contrast enhancements, histogram equalization. A particular combination of preprocessing steps can be applied to images in successive steps or in parallel to copies of the image.

A simplified example of a smoothing and background subtraction procedure in a MatLab language is presented in computer code below:

```
function Isubtracted = cmBackgrSubtrl(I,k)
% cmBackgrSubtrl(I,k) - simple flat background (=modal*k)
subtraction
% Y = cmBackgrSubtrl(I, k) - image Y is generated by
```

```

% subtraction (with saturation) of modal pixel value of I
multiplied by k
% DEFAULT - k=1
%
5  if (nargin == 1)
    k=1;
end
if (size(k)~=1)
    error('cmBackgrSubtrl: parameter k should be a number.
10  Exiting...');
end

%modpixnum = floor(size(I(:,1))/2);
%sortedval = sort( double(I(:)) );
15  %modpixel = sortedval(modpixnum);
    modpixel = median(double(I(:)));
    bg = k*modpixel;

    Isubtracted = mmsubm( uint8(I), uint8(round(ones(
20  size(I))*k*modpixel )) );

```

An example of a procedure for thresholding in computer code (MatLab) is presented below:

```

function thresh = GetThreshByPerim1(I, M)
25  % GetThreshByPerim1(I) Finds optimal thresholding value
    for image I
    % N = GetThreshByPerim1(I) Finds thresholding value N for
    image I
    % N = GetThreshByPerim1(I, M) - tests threshold values up
30  to M
    % DEFAULT M = maximum pixel value in I
    % note that GetThreshByArea is significantly faster
    % finds a threshold value that causes the maximal change
    in the

```

```
% total perimeter of the objects (Russ ????)
% see Matlab_Auto_threshold1_1-23-99.doc for more details
% Note: works somewhat better on SMOOTH images (i.e.
medfilt2(I, [3 3]) two times

5
if (nargin == 0)
    error (strcat( mfilename, ' : at least one parameter
required'));
elseif (nargin == 1)
10    M = double(max(I(:)));    %test thresholds up to
    maximum pixel value in I
    elseif (nargin > 2)
        error (strcat (mfilename, ' : too many parameters'));
    end
15
    if (size(M)>1)
        error (strcat(mfilename, ' : argument M should be a
number'));
    end
20
    Minval = double( min(I(:)));
    step = 1;

    %generate vertical vector perims with total perimeters of
25 objects at different
    %threshold values
    for i=Minval : step : M
        bwI = im2bw(I, i/255);
        prI = bwperim(bwI);
30    pr = sum(prI(:));
        if (exist('perims', 'var') == 0) %perims is yet
undefined
            perims = pr;
        else
```

```

        perims = cat(1, perims, pr);
    end
end

```

```

5  % vector prdiffs contains differences between successive
    perimeters
    prdiffs = diff(perims);
    mindecrease = min(prdiffs);
    minvalues = find(prdiffs == mindecrease);
10 index_of_mindecrease = minvalues(1);
    thresh = index_of_mindecrease + 1;

    % =====end GetThresh1=====

```

15           Thresholding provides a specific intensity, such that pixels darker than the threshold are deemed black, and pixels lighter than the threshold are considered white. The thresholded image can be processed using binary image processing techniques in order to extract regions.

(2). In a step 742+744, the digitized image input is subjected to object  
20 identification. This can be accomplished by a variety of procedures, for example by thresholding or edge detection and subsequent morphological opening and closing. Edge detection can be accomplished by means of gradient-based or zero-crossing methods, such as Sobel, Canny, Laplassian, Perwitt, and other methods.

An example of object identification procedure based on Canny edge  
25 detection (in MatLab language) is presented below:

```

function Imask = cmMaskDNA1(I);
% cmMaskDNA1 - generates binary mask for cell nuclei
through edge detection
30 % Imask = cmMaskDNA1(I)
% PARAMETERS
%   I - intensity image (grayscale)
% OUTPUT
%   Imask - BW image with objects from I

```

```

%
% For more details see Notebook Matlab_DNA_masking1_1-22-
99.doc
% Uses SDC Morphology Toolbox V0.7
5
if (nargin ~= 1)
    error('Wrong number of input parameters');
end
if (nargout ~= 1)
10    error('Wrong number of output parameters: one output
argument should be provided');
end

15  Imask = edge(I, 'canny');
    Imask = mmdil(Imask, mmsecross(1));
    Imask = mmero ( mmclohole(Imask,mmsecross(1)));
    Imask = mmedgeoff(Imask, mmsecross(1));
    % note that mmedgeoff this command removed FILLED OBJECTS
20  but not touching OUTLINES.
    % these outlines can be removed by filtering:
    Imask = medfilt2(Imask, [5 5]);

    %=====end cmMaskDNA1
25  =====

```

However, embodiments can also use other techniques, such as Fast Fourier Transforms (FFT) and the like as known in the art without departing from the scope of the present invention.

30 (3). In a step 746, a plurality of region features can be determined. For example, in a representative embodiment, image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Features not directly related to individual objects are also being extracted.



An example of a procedure for extraction of some of the features (MatLab language) is presented below:

```

function OData = cmGetObjectsData(I, Ilabel)
5  % cmGetObjectsData returns array measurements of objects
   in image "I" masked by "Ilabel"
   % EV 2-3-99; 2-10-99
   % OData = cmGetObjectsData(I, Ilabel) returns an array of
   morphological and intensity measurements
10  %   taken from a grayscale image "I". Objects are
   identified on a mask image Ilabel, usually
   %   created by bwlabel()
   % OUTPUT:
   % Each row in the output array OData represents
15  individual object
   % columns contain the following measurements:
   %
   %   1 - Index ("number" of an object);      8 -
   Solidity;
20  %   2 - X coordinate of the center of mass; 9 - Extent;
   %   3 - Y coordinate      -"-      ; 10 - Total
   Intensity;
   %   4 - Total Area (in pixels);              11 - Avg.
   Intensity;
25  %   5 - Ratio of MajorAxis/MinorAxis;        12 - Median
   Intensity;
   %   6 - Eccentricity;                        13 - Intensity of
   20% bright pixel
   %   7 - EquivDiameter;                       14 - Intensity of
30  80% bright pixel
   %
   % For details on morphological parameters see information
   on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

if (nargin ~= 2)
5   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
    error ('function has 1 output argument (array X by
14) ');
10 end

% finished checking arguments

% first collect morphological parameters in a structure
15 array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
'MajorAxisLength',...
    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
...
20 'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

25 %preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
30 for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
```

```
    OData(k, 5) = (ImStats(k).MajorAxisLength) /  
    (ImStats(k).MinorAxisLength);  
    OData(k, 6) = ImStats(k).Eccentricity ;  
    OData(k, 7) = ImStats(k).EquivDiameter;  
5    OData(k, 8) = ImStats(k).Solidity;  
    OData(k, 9) = ImStats(k).Extent;  
  
    % now collect and assign intensity parameters from  
    image I  
10  
    object_pixels = find( Ilabel == k);  
    object_area = size(object_pixels, 1); %same as total  
    number of pixels in the object  
    object_intensities = double(I(object_pixels)); %  
15    need to convert to double to do math  
    sorted_intensities = sort(object_intensities); %  
    will need to get median, 20% and 80% pixels  
    total_intensity = sum(object_intensities, 1);  
    avg_intensity = total_intensity / object_area;  
20    median_intensity = sorted_intensities( floor(  
    object_area/2 ) + 1 );  
    pix20 = sorted_intensities( floor(object_area*0.2)+1  
    ) ; %brightest pixel among dimmest 20%  
    pix80 = sorted_intensities( floor(object_area*0.8)+1  
25    ) ;  
  
    OData(k, 10) = total_intensity;  
    OData(k, 11) = avg_intensity;  
    OData(k, 12) = median_intensity;  
30    OData(k, 13) = pix20; %brightest pixel among dimmest  
    20%  
    OData(k, 14) = pix80; %dimmest pixel among brightest  
    20%  
end %for
```

```
%===== end function  
cmGetObjectsData() =====
```

5 (4). In a step 748, quantitative descriptors characterizing cell state are calculated based on the feature measurements extracted at step 746. For example, histogram distribution of intensities of cell nuclei provides information about the population cell cycle stages.

10 In a particular embodiment according to the present invention, data analysis techniques for describing the fluorescence patterns of cell portions in multiple cell lines in the presence and absence of compounds are provided. Automated image analysis techniques can include determining one or more regions from around nuclei, individual cells, organelles, and the like, called "objects" using a thresholding function. Objects that reside on the edge of an image can be included or  
15 excluded in various embodiments. An average population information about an object can be determined and recorded into a database, which can comprise a database text file or Excel spreadsheet, for example. However, embodiments can use any recording means without departing from the scope of the present invention. Values measured can be compared to the visual image. One or more types of numerical  
20 descriptors can be generated from the values. For example, descriptors such as a number of objects, an average, a standard deviation of objects, a histogram (number or percentage of objects per bin, average, standard deviation), and the like can be determined.

25 In a particular embodiment according to the present invention, data can be analyzed using morphometric values derived from any of a plurality of techniques commonly known in the art. For example, a software package called MetaMorph Imaging System, provided by Universal Imaging Corporation, a company with headquarters in West Chester, PA and NIH Image, provided by Scion Corporation, a company with headquarters in Frederick, Maryland.

30 Fluorescent images can be described by numerical values, such as for example, an area, a fluorescence intensity, a population count, a radial dispersion, a perimeter, a length, and the like. Further, other values can be derived from such measurements. For example, a shape factor can be derived according to a relationship

$4\pi * \text{area} / \text{perimeter}$ . Other values can be used in various embodiments according to the present invention. Such values can be analyzed as average values and frequency distributions from a population of individual cells.

In a particular embodiment according to the present invention, techniques for the automatic identification of mitotic cells are provided. Image analysis techniques employing techniques such as multidimensional representations, frequency-based representations, multidimensional cluster analysis techniques and the like can be included in various embodiments without departing from the scope of the present invention. Techniques for performing such analyses are known in the art and include those embodied in MatLab software, produced by MathWorks, a company with headquarters in Natick, MA.

Scalar values providing efficacious descriptors of cell images can be identified using the techniques of the present invention to perform predictive analysis of drug behavior. In a presently preferred embodiment, a plurality of heterogeneous scalar values can be combined to provide descriptors for each manipulation. By applying predictive analysis routines to the collections of these descriptors, predictive information about any number of manipulations and cell interactions can be extracted.

Fig. 7E illustrates a representative block flow diagram of simplified process steps for analyzing image feature values to obtain descriptors of cell state of step 718 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7E illustrates an input data of descriptors of known manipulations 319. A step 320 of reformatting and transforming data 319 to formats suitable for analysis is performed. Additionally, a "cleaning" process can eliminate outlying data points and the like in the data. Then, in a step 322, a decision is made whether to continue with step 324 or with step 326 based upon determining a particular type of analysis appropriate for the present application or particular type of prediction. If decisional step 322 determines processing should continue with step 324, then, in that step, an error estimate using a set of test descriptors is performed to estimate the quality of a prediction and processing continues with step 320. Once an optimal prediction is achieved, processing continues with step 326. In step 326, optimal transformation parameters and prediction methods are selected for use in

steps 328 and 330 which analyze data about an unknown manipulation. In a step 328, a solution is generated based upon any of techniques including training a neural network, solving a mathematical equation, applying decision tree rules and/or the like. In a step 330, an input data set of unknown descriptors 318 is reformatted and transformed based upon the optimal transformation parameters selected in step 326 using the transformation procedures in steps 320, 322 and 324. In a step 332, predictions techniques are applied to the reformatted manipulations from step 330 and the solution generated in step 328 and a plurality of properties of known manipulations 317 (e.g., therapeutic properties, and the like) in order to determine a prediction of properties of unknown manipulation 316.

Fig. 7F illustrates a representative block flow diagram of simplified process steps for a method of mapping a manipulation of cells to a physiological characteristic in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

- (1) In a step 750, a plurality of cells, e.g., dead, live, cell fractions or mixtures of cells are provided.
- (2) Then, in a step 752, the plurality of cells is manipulated, where manipulation occurs using a source(s) from one or a combination selected from an electromagnetic, electrical, chemical, thermal, gravitational, nuclear, temporal, or a biological source.
- (3) Next, in a step 754, a feature value is captured from the plurality of cells. The feature value can include one or any combination of characteristics such as cell count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface area, average intensity, total intensity, and optical density. This list is not meant to be limiting.
- (4) Then, in a step 756, a degree of presence of one or more feature values is assigned for each manipulation.
- (5) In a step 758, the feature values from the plurality of cells are stored in memory locations. From the memory locations the values can be used for

statistical analyses to produce predictive information about the relatedness of the descriptors of the manipulations to one another. This information is used to infer properties of the manipulations.

Fig. 7G illustrates a representative block flow diagram of a simplified process steps for a method for populating a database with manipulated biological cell information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 760, a plurality of cells in various stages of the cell cycle, A montage image that was used as a source to generate data in Appendix A is presented in Fig. 12., such as for example, the stages of interphase, prophase, metaphase, anaphase, and telophase are provided.

(2) Then, in a step 762, each of the cells in the various stages of mitotic development is manipulated.

(3) Next, in a step 764, an image of the plurality of manipulated cells is captured using image acquisition techniques in order to provide a morphometric characteristic of each of the manipulated cells.

(4) As a preferable option, in a step 766, an image database may be populated with the image of the plurality of manipulated cells.

(5) Following step 764 or optional step 766, a morphological value is calculated from the image in a step 768.

(6) In a step 770, the database is populated with the morphological value.

Fig. 7H illustrates a representative block flow diagram of simplified process steps for a method for populating a database with manipulated biological information, e.g., image acquisition parameters, image feature summary information, and well experimental parameters in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7H illustrates a step 780 in which cells are placed into site on a plate and a manipulation is applied. Then, in a step 781 an image is taken of the cells. In step 782, the image is transferred to an image archive

database. Then, in a step 783, well experimental parameters are entered into the database 787. Well experimental parameters can include cell type, manipulation and the like. In a step 784, image acquisition parameters are transferred to database 787. Image acquisition parameters can include file name, fluorophores and the like. In a  
5 step 785, the image acquired in step 781 is analyzed. Then, in step 786, an image feature summary from the analysis step 785 is transferred to database 787.

In step 788, a lookup table for all analyses is provided to database 787. The lookup table provides information about the analyses. In a step 789, a query of database 787 for process data is performed. The results are reformatted. Then in a  
10 step 790, the database 787 is queried. Next, in a step 791, features of the manipulations stored in the database are combined and reduced. Next, in a step 793, reduced features of step 791 can be compared. In a step 792, the results of step 793 are recorded in database 787. Then, in a step 794, a report of predictions based on comparisons performed in step 793 is generated.

15 Fig. 7I illustrates a representative block flow diagram of simplified process steps for acquiring images of manipulated biological information, e.g., cells, cell tissues, and cell constituents in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,  
20 modifications, and alternatives. Fig. 7I illustrates a step 770 in which a user sets up an image analysis procedure. Then, in a step 772, an image is read into image analysis software. Next, in a step 774, patterns and objects are identified in the image using one or more algorithms. Next, in a step 776, sets of features are extracted from the image. Then, in a step 778, feature information, descriptor values and the like are  
25 exported to the database, such as database 787 of Fig. 7H, for recording. Next, in a decisional step 779, a determination is made whether any more images should be taken. If this is so, processing continues with step 772. Otherwise, image acquisition processing is completed.

Fig. 7J illustrates a representative block flow diagram of simplified  
30 process steps for populating, acquiring and analyzing images of manipulated biological information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,



modifications, and alternatives. Fig. 7J illustrates a step 300 of placing a plate onto an imaging stage and reading a bar code. Then, in a step 301 an autofocus procedure is performed. Next, in a step 302, a first optical filter configuration is selected and an image is collected. Then, in a decisional step 303, a determination is made whether more than one image per optical configuration can be taken. If so, then, in a step 304, a new position within the well is targeted and another image is collected. Then, in a decisional step 305, a determination is made whether any more images need to be collected. If this is so, step 304 is repeated until all images for a particular well have been collected. After one or more images are collected for the well, in a step 306, the stage is returned to a starting position within the well, and a montage is created from collected images. The results are named with a unique file name and stored.

In a decisional step 307, a determination is made whether any more optical channels in the well can be imaged. If this is so, then in a step 308 the next optical filter configuration is selected and an image is collected. Processing then continues with decisional step 303, as described above. Otherwise, if no further optical channels in the well can be imaged, then in a decisional step 309 a determination is made whether any wells remain to be imaged. If not all wells have been imaged, then in a step 310, the stage moves to the next well and processing continues with step 301, as described above. Otherwise, if all wells on the plate have been imaged, then in a decisional step 311, a determination is made whether any more plates can be processed. If this is so, then processing continues with step 300 as described above. Otherwise, in a step 312, the information is stored to a CD or other storage device as a backup.

Fig. 7K illustrates a representative block flow diagram of simplified process steps compound based upon information about effects of one or more known compounds on a cell population in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7K illustrates a step 340 of populating a database with descriptors for known compounds. Such descriptors can be determined from imaging the cell population. However, in some embodiments, descriptors can be derived by measurements and combinations of measurements and the like. Then, in a step 342, descriptors for the unknown compound are determined from imaging a

second cell population. The second cell population has been treated with the unknown compound. Then, in a step 344, a relationship between the descriptors determined from the unknown compound with the descriptors determined from the known compounds can be determined. Finally, in a step 346, an inference can be made about the unknown compound based upon the descriptors of the known compounds from the relationship determined in step 344.

Accordingly, the present invention provides a novel database design. In a particular embodiment according to the present invention, a method for providing a database comprises measurement of a potentially large number of features of one or more sub-cellular morphometric markers. Markers can be from any of a large variety of normal and transformed cell lines from sources such as for example, human beings, fungi, or other species. The markers can be chosen to cover many areas of cell biology, such as, for example markers comprising the cytoskeleton of a cell. The cytoskeleton is one of a plurality of components that determine a cell's architecture, or "cytoarchitecture". A cytoarchitecture comprises structures that can mediate most cellular processes, such as cell growth and division, for example. Because the cytoskeleton is a dynamic structure, it provides a constant indication of the processes occurring within the cell. The cytoarchitecture of a cell can be quantified to produce a one or more scalar values corresponding to many possible cellular markers, such as cytoskeleton, organelles, signaling molecules, adhesion molecules and the like. Such quantification can be performed in the presence and absence of drugs, peptides, proteins, anti-sense oligonucleotides, antibodies, genetic alterations and the like. Scalar values obtained from such quantification can provide information about the shape and metabolic state of the cell.

In a presently preferred embodiment, scalar values can comprise morphometric, frequency, multi-dimensional parameters and the like, extracted from one or more fluorescence images taken from a number of cellular markers from a population of cells. Two or more such scalar values extracted from a plurality of cell lines and markers grown in the same condition together comprise a unique "fingerprint" or descriptor that can be incorporated into a database. Such cellular descriptors will change in the presence of drugs, peptides, proteins, antisense oligonucleotides, antibodies or genetic alterations. Such changes can be sufficiently unique to permit a correlation to be drawn between similar descriptors. Such

correlations can predict similar properties or characteristics with regard to mechanism of action, toxicity, animal model effectiveness, clinical trial effectiveness, patient responses and the like. In a presently preferred embodiment, a database can be built from a plurality of such descriptors from different cell lines, cellular markers, and compounds having known mechanisms of action (or structure, or gene response, or toxicity).

The present invention also provides database and descriptor comparisons according to other embodiments. In a particular embodiment according to the present invention, measurement of scalar values or features can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be compared using a plurality of techniques, such as a technique of creating "phylogenetic trees" of a statistical similarity between the descriptors from various drugs. In a present embodiment, scalar, numeric values can be converted into a nucleotide or amino acid letter. Once converted into a corresponding nucleotide representation, the descriptors can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. In an alternative embodiment, numeric values for the fingerprints can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound fingerprint with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphometric properties and features of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, a clustering can be performed on acquired image descriptors. Some embodiments can comprise statistical and neural

network - based approaches to perform clustering and comparisons of various descriptors. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data. In some embodiments, clustering and comparing can be performed on features extracted from cell images. In a presently preferable embodiment, procedures for comparisons and phylogenetic analysis of biological sequences can be applied to data obtained from imaging cells.

Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the program Phylip, available at <http://evolution.genetics.washington.edu/phylip.html>, and other packages listed at <http://evolution.genetics.washington.edu/phylip/software.html>. However, select embodiments according to the present invention can comprise a technique of statistical classification, statistical clustering, distance based clustering, linear and non-linear regression analysis, self-organizing networks, and rule-based classification.

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes. Resulting "pseudo-sequences" can be subjected to analysis by a sequence comparison and clustering program.

Other types of databases can also be provided according to other embodiments. The database includes details about the properties of a plurality of standard drugs. When the descriptor of a test compound is compared to the database, predictions about the properties of the test compound can be made using any known property of the other compounds in the database. For example, properties about a compound in the database could include structure, mechanism of action, clinical side effects, toxicity, specificity, gene expression, affinity, pharmacokinetics, and the like. The descriptor of a compound of unknown structure from a natural products library could be compared to the descriptors of compounds with known structure and the structure could be deduced from such a comparison. Similarly, such information could lead to better approaches to drug discovery research including target validation

and compound analogizing, as well as pre-clinical animal modeling, clinical trial design, side effects, dose escalation, patient population and the like.

According to the present invention, databases can be integrated with and complementary to existing genomic databases. Differential genomic expression strategies can be used for drug discovery using database technology. In one particular embodiment, cell data and cellular response data can be associated with a genetic expression profile assay to form a single assay. Live cells expressing fluorescence markers can be treated with a drug, imaged and analyzed for morphometry; and then analyzed for mRNA for expression. Such embodiments can provide rapid development of tools to link cellular behavior with functional genomics.

Database methods according to the present invention can be used to predict gene function and to assist in target validation. Databases that include genetic diversity, i.e., having cellular descriptors from cells of differing genetic backgrounds (tumor, tissue specific, and gene knock out cell lines), can provide the capability to compare cells of unknown genetic background to those in the database. Similarly, the descriptor of an unknown cellular portion in the presence of multiple drugs can be queried against the descriptors of the known markers in the database. For example, if an unknown gene is tagged with Green Fluorescent Protein (GFP), the database may be used to identify the cellular portions for which that unknown gene encodes.

According to the present invention, target validation and specialized cell-based assay screening can be performed using database systems and methods to serve as a universal high-throughput cell-based assay that can evaluate the molecular mechanism of drug action. As new genes are isolated and identified, a large collection of available gene-based knowledge is becoming available. From this large collection of new genes, potential protein targets can be identified using the genomic tools of sequence analysis and expression profiling. However, unless a gene mutation is tightly linked to a disease state, further validation of individual targets is a time consuming process, becoming a bottleneck in drug discovery. Furthermore, robotics and miniaturization are making "High Throughput Screening (HTS)" the industry standard, substantially reducing the time and cost of running a target-based biochemical assay. Therefore, it is now possible to routinely screen large libraries and use a resulting "hit" to validate the target. In such approaches, a specialized cell-based assay would be developed to test hits for each target. Since this often involves

the creation of cell lines expressing new markers, this stage may also become a bottleneck that cannot keep pace with HTS. In addition, these cell-based assays may not be amenable to high-throughput screening, making it difficult to test the increasing number of analogs arising from combinatorial chemistry.

5           In a particular embodiment according to the invention, a rapid characterization of large compound libraries for potential use as pharmaceutical products can be provided by predicting properties of compounds that relate to the compounds' potential as bioactive drugs. In many drug discovery situations, virtually millions of compounds can be passed through a HTS assay against a small number of  
10   validated targets. These assays produce hundreds to thousands of potential hits. These hits can then be subsequently screened by a pipeline of secondary and tertiary screens to further characterize their specificity, often time completely missing non-specific interactions with other proteins. Techniques according to the present invention can provide a replacement to such screening operations by providing  
15   information about cellular accessibility and mechanism of action for the hits coming from a HTS system. Furthermore, it can replace the biochemical HTS assay and allow rapid and accurate identification of attractive compounds from large libraries without an intervening biochemical assay. The cell information can be predictive of whether to continue into an animal model for each compound, and which animal model to  
20   pursue.

          The principles of the present specifically contemplate a wide variety of research methodologies, or usage scenarios, implementing these principles. The following discussion of three such scenarios is by way of illustration and not limitation. Study of the principles enumerated herein will render evident to those  
25   skilled in the art certain additional methodologies or usage scenarios enabled by the teachings hereof. The present invention specifically contemplates all such modifications. The following description presents some specific embodiments and scenarios that represent a broader use of cellular phenotypic data and characterizations to deduce mechanisms of action and other features of cellular  
30   responses to various stimuli. Such procedures generally involve producing a quantitative cellular phenotype based upon two or more cellular attributes and then comparing that phenotype to phenotypes previously stored and indexed. Such

procedures make use of databases or other repositories of biological information. The invention is not limited to the specific embodiments described here.

Considering first the procedure 2000 depicted in Figure 20, a compound has been identified as having a particular cellular activity. See 2004. For example, a compound may be found to inhibit the growth of certain cancer cell *in vitro* by a specific and desired mechanism of action. This may be a particular company's "gold standard."

Next, the compound is analyzed at 2006 in terms of its effect on one or more cell lines. More specifically, the compound is linked, virtually, to a particular phenotype. Two or more values or measures of cellular attributes characterize that phenotype. These attributes are quantified in the context of specific cellular markers.

In one example, the cellular marker is an organelle such as a nucleus or Golgi apparatus. Measured attributes useful for characterizing an associated phenotype include geometric parameters (e.g., size, shape, and/or location of the organelle) and composition (e.g., concentration of particular biomolecules within the organelle).

The phenotype may be characterized by administering the compound of interest to various cell lines and in various concentrations. In each example within this matrix, the attributes of interest are measured. Ultimately, certain phenotypic features (combinations of attribute values) are associated with the compound of interest. These features provide a template for the phenotype.

Next, using the phenotype as identified at 2006, the process identifies other compounds providing similar features. The goal here is to present a list of compounds having a mechanism of action similar to that of the compound that started the process. This allows researchers to identify a mechanism of action, if not already known, for their compound and to draw conclusions based upon their compound's link to other known compounds (which may not be chemically/structurally similar to the compound of interest).

Identifying similar compounds based upon phenotype can take many paths. Most will involve some mathematical basis. For example, the phenotype defined at 2006 can be represented as a fingerprint or vector comprised of multiple scalar values of cellular attributes (as described above). The phenotype representation can then be compared against known phenotypes characterized by the same format

(e.g., they are all characterized as vectors having the same attribute set, but with different values of the attributes). The comparison may be as simple as a Euclidean distance or more sophisticated as a neural network or multivariate statistical correlation.

5                   The known compounds and associated phenotypes may be stored as database records or other data structures that can be queried or otherwise accessed as part of the identification procedure. The compounds may also be associated with other relevant data such as clinical toxicity, cellular toxicity, hypersensitivity, mechanism of action, etc. (when available).

10                   Compounds found to be sufficiently similar to the starting compound are returned for consideration by researchers. A data processing system may rank such compounds based on degree of similarity to the starting compound. In some cases, the system may even provide similarity scores associated with the listed compounds.

15                   Often researchers wish to determine whether their particular compound has clinical or biochemical effects beyond those that they are already aware of. In a typical scenario, the compound of interest was selected based upon its strong binding a target or its stimulation or inhibition of cell growth in a particular cell line. The process associated with 2010 has likely identified the compound of interest as having  
20                   a particular mechanism of action based on phenotypic similarity to other compounds having a similar mechanism of action. However, within the region of biochemical space, there may be subspaces (characterized by subphenotypes) that correspond to separate properties. For example, within the phenotypic space associated with one mechanism of action, there may be subspaces associated with clinical toxicity,  
25                   cellular toxicity (likely overlapping the clinical toxicity space), and little or no toxicity. Obviously, a researcher would like to know whether her compound is likely to be toxic.

                  Thus, the process 2000 may include characterizing the compound of interest in terms of its distance from (i.e., similarity to) specific phenotypes having  
30                   known characteristics. In a typical example, the known characteristic is toxicity. This feature allows the researcher to quantify her compound in terms of mechanism of action AND toxicity (or in terms of two or more other relevant properties associated



with phenotype). To allow simple ranking or characterization, compounds of interest may be scored according to a simple or weighted Boolean expression.

A second scenario of interest is depicted in Figure 21. This scenario again defines a phenotype in terms of a quantifiable vector or other measure.

5 However, rather than using a compound of interest to generate the phenotype, some other cellular stimulus is used to generate the phenotype.

As shown, a process 2100 begins with receipt of cells of interest. See 2104. In many situations, the cells are produced by a genetic or epigenetic process that affects the expression level or activity of a particular protein. More generally,  
10 any cellular stimulus (e.g., radiation level and type, gravity level, magnetic field, acoustic perturbations, etc.) can be used to generate the cell line of interest. Importantly, this stimulus affects the phenotype and can be correlated therewith.

In the context of drug discovery, a gene encoding for a particular target can be genetically knocked out, underexpressed, overexpressed, expressed in a non-  
15 native state, etc. This may be accomplished via standard procedures involving genomic modification, translation or transcription apparatus modification (e.g., use of antisense nucleic acids), blocking target activity (using antibodies to a receptor site for example), and the like. These processes will generally affect the phenotype in some quantifiable way. Importantly, they clearly and unambiguously define a cellular  
20 phenotype associated with altering the activity of the target protein.

At 2106, the process involves measuring one or more cellular features from the cell line of interest to define/quantify the phenotype. This may be accomplished as described above with reference to 2006. Next, at 2108, the cellular phenotype generated in this manner is used to identify and rank a set of compounds  
25 associated with the phenotype. This operation may proceed in the manner of operations 2008 and/or 2010 from Figure 20.

Finally, at 2110, the process clusters the compounds returned at 2108 by a mechanism of action. The operation 2106 has tightly bound a mechanism of action to a phenotype. Various compounds characterized and stored in a system  
30 database may be tentatively assigned a mechanism of action or may have no suggested mechanism of action. By matching their virtual phenotype to the phenotype generated at 2106, one can create or strengthen an association between the compounds and mechanism of action relevant to the stimulus at 2104.

Considering now Figure 22, a third scenario is depicted. This scenario again involves using a virtual phenotype to glean information relevant to a mechanism of action or other cellular activity. In this case, assay data from a group of compounds (e.g., a primary or focused library) is used to elucidate a phenotype.

5           As shown, a process 2200 begins by identifying a target protein. See 2204. Then, at 2206, the process involves identifying positive and negative biochemical hits. More generally, this may involve ranking a number of compounds based upon their interaction with the target. In a specific case, the compounds are ranked based upon their binding affinities to or ability to inhibit the enzymatic activity  
10 of the target protein.

After the compounds have been characterized in some manner based upon their interaction with the target, they are used to define a cellular phenotype. See 2208. Generally, the techniques to accomplish are the same as described with reference to operation 2006 of Figure 20. In this case however, one may obtain a  
15 strong correlation between mechanism of action (involving the target) and phenotype by using multiple of the compounds identified at 2206. For example, some of the "best hits" may be administered to cell lines in various concentrations. And some of the least effective compounds may also be administered. Cellular attributes that are more strongly exhibited with increasing concentration of the best hits (and not  
20 exhibited or exhibited only weakly upon administration of the negative hits) can be used to define the virtual phenotype. In a related approach, compounds having widely varying levels interaction with the target are administered to cells. Those cellular attributes that vary linearly or at least monotonically with the degree of interaction between the target and compound represent attributes that can be used to define the  
25 virtual phenotype.

After the cellular phenotype has been defined, previously characterized compounds may be clustered with that phenotype. See 2210. As with operation 2110 of Figure 2, this may create or strengthen an association between a mechanism of action and various compounds in a database.

30           Finally, and optionally, procedure 2200 may provide a "higher resolution" mechanism of action for the compounds identified at 2206. See 2212. Presumably interaction with the target suggests a specific mechanism of action or at least some aspect of a mechanism of action. However, a given target may participate

in a larger cellular mechanism of action – unknown to researchers. Further, a compound may that binds with the target may participate in multiple mechanisms of action – some of which do not involve the target. By linking the target (and its positive hits) to a particular phenotype, some of these additional cellular level activities can be elucidated. The defined phenotype may have been previously identified as associated with other mechanisms of action or higher resolution mechanisms of action. Thus, the phenotype identified at 2208 can be leveraged to generate a higher resolution mechanism of action at 2212.

As suggested in the above discussion, compounds and associated phenotypes may be stored as database records. Such databases can take on many flavors. In one example, a database includes various pieces of information relevant to oncology. Such database may include numerous compounds classified by cellular phenotype, mechanism of action, toxicity, etc. More specifically, the database may include data on commercially available compounds clustered by cellular phenotypes corresponding to mechanisms of action. Further the databases of interest may extended or combined (via standard relational tables and algebra for example) to include additional data such as pharmacology data, cellular genomics data, gene expression data, protein expression data, etc. In a specific example, the database includes measurements made on a subset of the NCI60 cell lines, using DNA, Golgi apparatus, and/or microtubules as markers for defining the phenotypes. Other data includes dosage response information, variation in effect over time, etc. The compounds populating the database could include known National Cancer Institute oncology study compounds. In a specific embodiment, the compound set includes some or all of the compounds mentioned in the article “A gene expression database for the molecular pharmacology of cancer,” Nature Genetics, 24, pp. 236-244 (March 2000).

Various biological analyses may be conducted to develop additional information for characterizing compound mechanisms of action, etc. For example, a cell count analysis may be used to develop dose response curves, GI 50 data, etc. The cell cycle may also be analyzed to find out how various stages in the cycle vary in response to particular stimuli. The Golgi apparatus may be analyzed to determine whether it is in a normal state, a dispersed state, a diffused state, etc. As another example, tubulin may be analyzed to determine whether it is normal, de-polymerized,

over-polymerized, bundled, etc. Obviously, combinations of such analyses may be performed. For example, properties of the Golgi apparatus or tubulin may be analyzed over one or more cell cycles.

5 In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs, such as clinical trial and patient response information, will be used in a similar fashion as the pre-clinical information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions  
10 will be able to provide predictive value for this aspect of drug development.

Although the above has generally been described in terms of specific hardware, software, and methods, it is understood that many alternatives can exist. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the  
15 workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives. Some examples according to the present invention are provided below.

20

## EXPERIMENTS

To prove the principle and demonstrate the objects of the present invention, experiments have been performed to determine the effects of manipulations on cell structure using imaging and analysis techniques applied to a variety of  
25 situations. These experiments were performed by growing multiple cell lines in the presence of multiple compounds, or substances. Cells were fixed and stained with fluorescent antibodies or labels to multiple cellular portions. One or more images of the cells were then obtained using a digital camera. Descriptors were built by quantifying and/or qualifying patterns of one or more feature from each image in the  
30 cell lines under study. A database was built from the descriptors. As the database grows, it should be able to predict the mechanism of action of an unknown drug by comparing its effect with the effects of known compounds or to identify data clusters within large libraries of compounds.

In a first experiment, an automated method to count the number of cells and differentiate normal, mitotic, and apoptotic cells was created.

Approximately, 5,000 HeLa cells were plated per well in a 96 well plate and grown for 3.5 days. The cells were fixed with  $-20^{\circ}$  MEOH for 5 minutes, washed with TBS for 15 minutes, and then incubated in 5 mg/ml Hoechst 33342 in TBS for 15 minutes. Then, 72 images were collected with a 40x objective and 75 ms exposure time.

The analysis was performed on objects that met a certain size criteria that was based on 1) measuring the size of objects in the image that were clearly not cells and 2) excluding the first peak of the area histogram (Fig. 8B values 1-4654).

Histograms of the individual object data were generated for each type of feature. Fig. 8A shows the histogram for average intensity, and Fig. 8B shows histogram data for the area of each object. Fig. 8C shows the scatter plot of the average intensity vs. the area of all of the objects. The pattern of the scatter plot showed an interesting pattern: a large cluster of cells in one region of the graph, with a scattering of object points in other regions. Because mitotic structures are identified as particularly bright objects, most likely due to the biological fact that the chromatin is condensed, the original Hoechst images could be used to identify which cells were either undergoing mitosis, or otherwise looked abnormal. Manual inspection of 917 cells resulted in the classification of each object. Fig. 8D shows a graph where each type of cellular classification is delimited. This graph clearly shows that the mitotic nuclei are brighter than the interphase nuclei. Further, the different phases of the cell cycle can be separated using these two features. Figs. 8E-8F show bar graphs of the average and standard deviations of the areas and average intensities for each cell classification type. These graphs show that interphase nuclei are statistically less bright than mitotic nuclei and that telophase nuclei are statistically smaller than other mitotic nuclei.

Each image was thresholded to an intensity level of 20. A standard area value was set at 9500 pixels. Automated information gathering about all of the objects was done and collected into an Excel spreadsheet (for more information see, section on imaging system). The following information was recorded:

IMAGE NAME
OBJECT #

AREA
STANDARD AREA COUNT
PERIMETER
FIBER LENGTH
FIBER BREADTH
SHAPE FACTOR
ELL. FORM FACTOR
INNER RADIUS
OUTER RADIUS
MEAN RADIUS
AVERAGE INTENSITY
TOTAL INTENSITY
OPTICAL DENSITY
RADIAL DISPERSION
TEXTURE DIFFERENCE MOMENT
EFA HARMONIC 2, SEMI-MAJOR AXIS
EFA HARMONIC 2, SEMI-MINOR AXIS
EFA HARMONIC 2, SEMI-MAJOR AXIS
ANGLE
EFA HARMONIC 2, ELLIPSE AREA
EFA HARMONIC 2, AXIAL RATIO
EFA HARMONIC 3, SEMI-MINOR AXIS

The following results were obtained:

- 1,250 objects were counted
- 201 of those objects has standard area counts  $> 2$  (area  $> 19000$  pixels)
- 195 objects had areas  $< 6000$  pixels
- 1529 objects estimated in total
- 1328 object areas are  $> 6000$  pixels
- The data was reduced to 917 objects that were  $6000 < \text{area} < 19000$
- For the 917 objects a scatter plot of area vs. average intensity and a histogram of the average intensity were generated.

- 116 objects that had average intensity intensities  $> 60$  were manually looked at to determine their morphology.

- Of those 116 objects:

6 were dead or indistinguishable

4 were interphase

30 were prophase

32 were metaphase

24 were anaphase

20 were telophase (10 pairs)

- 12 prophase objects were missed because of gray scale cut off. (8 of those prophase cells had gray scale values  $> 57$ , as did 7 interphase)

- 1 telophase object was missed because it was too small ( $< 6000$ )

- 1 prophase object was missed because it was too big ( $> 1900$ )

- 16 mitotic objects were missed because they were parts of objects with standard count  $> 2$ .

In sum, out of 917 single objects, the analysis correctly identified 106 out of 130 mitotic objects, or (81% predictive, 91% of identified mitotics). Out of 917 single objects, the analysis incorrectly identified only 10 non-mitotics as mitotics (1% total, 8% of identified mitotics); 14 mitotics as interphase (1.4% total, 1% interphase). An automated classification system that would automatically assign values to each object using these or other measurement features can thus be developed, utilizing the principles set forth herein.

In a second experiment, the effects of Taxol on MDCK cells and the different types of morphological effects were observed. A plurality of MDCK cells grown in 96 well plates were treated with Taxol for 4.5 hours at different concentrations (10 uM-1pM). They were then fixed, labeled with Hoechst, and imaged.

This experiment used a labeling protocol comprising: MEOH fix at – 20°, Wash in PBS, Block in PBS/BSA/Serum/Triton-X 100, Incubate with 5 µg/ml Hoechst 10 minutes, and wash.

Cells were inspected for different morphologies and manually counted at each different drug concentration in one well. Fig. 9 shows example images from each drug concentration and the different types of morphologies and cells are highlighted. Fig. 10 shows the distribution of each morphology within the cell population as a function of drug concentration. The higher the concentration of Taxol, the larger proportion of cells underwent apoptosis, and the fewer number of normal mitotic cells were detected.

In a third experiment, the purpose was to determine whether the automated analysis methods developed in the first experiment can detect differences in Hoechst morphology in the presence of 6 known compounds at one concentration and exposure time in one cell line. In this experiment, HeLa cells were separately treated with 6 compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black-walled 96 well tissue culture treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of cytochalasin D (CD), Taxol, hydroxyurea, vinblastine, nocodazole, and staurosporine was added to different wells at a 1:100 addition in DMSO.



The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. Then, 9 images per well were collected of the Hoechst staining using a 10x objective.

5 The low magnification images taken of Hoechst were run through the automated image analysis method described in the first experiment. Plots of the average intensity and area were made of each compound. Fig. 11 shows the scatter plots of the compounds. The scatter plots of each compound are visually distinct. For example, cells treated with CD are smaller than control, and cells treated with Hydroxyurea are larger and brighter. Furthermore, the number of cells per well was  
10 very different (data not shown).

The effects of different compounds can be clearly and automatically distinguished by identifying changes in cellular morphology. This method can also be used to count adherent cells.

The next experiment was to develop clustering algorithms that assign  
15 statistically meaningful values to the representative two dimensional data shown in Fig. 10, and even more complicated clustering of all of the multidimensional data that can be extracted across one, and multiple images.

A fourth experiment was performed to obtain high magnification images of two markers in the presence of drugs. In this experiment, HeLa cells were  
20 treated with 80 generic compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black walled 96 well tissue culture-treated plate and left to recover in the incubator for 24  
25 hours. After this time, 10 ug/mL of each compound from the Killer Plate from Microsource Discovery Systems (Gaylordsville, CT) was added to different wells at a 1:100 addition in DMSO. The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. In addition to being labeled with Hoechst 33342 (against chromatin),  
30 cells were also labeled with 1 unit of rhodamine-conjugated phalloidin (against actin) for 30 minutes.

The 96 well plate was imaged twice. Once, 9 images per well were collected of the Hoechst staining using a 10x objective. After this, one image per well of both the phalloidin and Hoechst staining was collected using a 40x objective.

The resulting high magnification images were analyzed qualitatively and distinct pattern differences were detected in both the Hoechst and phalloidin images. Fig. 12 shows three example images from the experiment. The top row is the Hoechst staining, and the bottom row is the phalloidin staining from the same well. The columns show the images from wells treated with just DMSO (control), cytochalasin D, and Colchicine. The morphology of each marker is different in the presence of each drug. Interestingly, there is an effect in the morphology of the chromatin in the Hoechst image of cytochalasin D, which directly targets the actin cytoskeleton (and thus there is an expected effect in the phalloidin image). Also, there is an effect on the actin cytoskeleton, compared to control, in the presence of colchicine that directly targets the microtubule network.

The low magnification images were analyzed as described in the first experiment, and different patterns were seen in both the average intensity vs. area plots, and in the number of cells per well (data not shown). Thus, changes in patterns of a marker that is "down-stream" from the direct target of a compound are detectable. Automated image analysis protocols for actin and other markers can be developed similarly, again utilizing the principles set forth herein.

A fifth experiment was performed to test quadruple labeling of 9 different cell lines grown in normal conditions. In this experiment, NCI-H460, A549, MDA-MD-231, MCF-7, SK-OV-3, OVCAR-3, A498, U-2 OS, and HeLa cells were plated. Then, the cells were fixed and stained for portions of the each cell known as DNA, tubulin, actin, and Golgi.

The following table summarizes the procedures for this experiment:

Action	Active Ingredient/Notes	Buffer	Vol/ well	Desired Time	Temp
Remove media	NOTE: gently by pipetting, not aspiration				
Fix	4% Formaldehyde	PBS	100µl	20 min	rt
Wash		TBS	100µl	5 min	rt
Wash		TBS	100µl	5 min	rt

Permeablize	0.1% Triton X-100	TBS	100 $\mu$ l	10 min	rt
Permeablize	0.1% Triton X-100	TBS	100 $\mu$ l	10 min	rt
Block	% BSA % Serum Filter sterilize before use	TBS w/azide	100 $\mu$ l	1hr or o/n	rt or 4°C
Primary Antibody	1:1000 dilution of DM1 $\alpha$	TBS + 1% BSA + 0.1% TX-100	50 $\mu$ l	1hr -- or o/n	rt or 4°C
Wash		TBS	100 $\mu$ l	5 min	rt
Wash		TBS	100 $\mu$ l	5 min	rt
Wash		TBS	100 $\mu$ l	5 min	rt
Fluorescent Stain	FITC lens culinaris 1:500 Rhodamine-Phalloidin 1:500 CY5 goat anti-mouse 1:100	TBS + 1% BSA + 0.1% TX-100	50 $\mu$ l	1 hr.	rt, dark
Wash		PBS	100 $\mu$ l	5 min	rt, dark
Hoechst	1:1000 dilution of 5mg/ml	TBS	100 $\mu$ l	15 min	rt, dark
Wash		PBS	100 $\mu$ l	5 min	rt, dark
Wash		PBS	100 $\mu$ l	5 min	rt, dark
Wash		PBS	100 $\mu$ l	5 min	rt, dark
Store		PBS	200 $\mu$ l	1 month	4°C

Cells were plated out at different densities for 48 hours. Cells were fixed and labeled by the above method. Cells were imaged using an automated imaging system that collected 9 images from each marker using a 10x objective.

Higher magnification images were collected of a few cells for demonstration purposes.

In this experiment, each cell line demonstrated different morphological patterns as determined by phase. For example, A549 cells are much more compacted than OVCAR-3 cells as determined by phase contrast imaging (data not shown). The different fluorescent markers showed even bigger differences between different cell lines. Figs. 13 and 14 show 4 panels of each marker for A549 (Fig. 13) and OVCAR-3 cells (Fig. 14). The markers are Hoechst (upper left), Phalloidin (upper right), Lens culinaris (lower left), and DM1a antibody (lower right). The following table summarizes the qualitative differences between these images:

MARKER	A549	OVCAR3
Hoechst/DNA	small	large
Phalloidin/actin	fuzzy	crisp - many stress fibers
Lens culinaris Golgi	compact	Disperse/punctate
DM1 alpha Tubulin	perinuclear	evenly distributed

Higher magnification images were taken of the OVCAR3 cells. Fig. 15 shows the same markers at 20x, and Fig. 16 shows the markers at 40x. While the highest magnification images show the most detail, these images illustrate that very little morphological or feature information is lost in the 10x images.

These data exemplify the differences in morphology seen between different cell types. Thus the automated image analysis software can be customized for each marker in each cell type. Different drugs should effect these morphologies differentially.

An automated quantification method for each marker and cell line can be similarly developed.

A sixth experiment was conducted with a more sophisticated software package and to develop more flexible image recognition algorithms. In this experiment, prototype image features extraction was performed using MatLab programming language with image toolbox and SDC morphology toolboxes. Algorithms are being developed that will automatically identify objects on images and

to measure various morphological and feature parameters of these objects. Many different features for each of the cellular markers were acquired.

An example of a MatLab program called "AnalyseDNA" that takes as an input an unlimited number of images, identifies individual objects in these images  
5 based on either their intensities, or based on edge-detection algorithms, and extracts a number of morphological and intensity characteristics of these objects. A copy of this program follows:

Listing of the AnalyseDNA.m program and of some of the supporting subroutines

10

```
function files_analysed = AnalyseDNA(filemask, outpath,  
nx, ny, filter_range, dext, modifier, sfname)  
% AnalyseDNA performs measurements on files of DNA images  
% V1. EV 2-11-99; 2-15-99; 2-16-99
```

15

```
%  
% files_analysed = AnalyseDNA(filemask, outpath, nx, ny,  
filter_range, dext, modifier, sfname)  
%  
% PARAMETERS:
```

20

```
% ALL PARAMETERS ARE OPTIONAL  
%
```

```
% FILEMASK - mask for file names to be analyzed  
INCLUDING PATH(for example c:\images\*.tif)
```

25

```
% DEFAULT '.*.tif' (all *.tif files in the current  
directory).
```

```
%
```

```
% OUTPATH - path to a directory where all the output  
files will be placed.
```

```
% DEFAULT - output is saved in the same directory
```

30

```
which contains images
```

```
%
```

```
% NX, NY - number of individual images in montage  
images along X and Y axes (DEFAULT 1)
```

```
%
```

```
%    FILTER_RANGE - 3 col-wide array (or[]). Specifies
how data is filtered when summary is calculated
%    this parameter internally is passed to GetDNADData
and then to GetSummaryData - see these
5 %    functions for details. For example: [2 2 Inf; 6 100
8000] will case all raws of data for which
%           values in column 2 are less than 2 and all raws
where values in column 6 are less than 100 or
%           more than 8000 to be excluded from all
10 calculations of a summary.
%    DEFAULT - [] (means do not filter, summarize all
data)
%
%    DEXT - string. Extension for data files being saved.
15 %    DEFAULT 'dat';
%
%    MODIFIER - this modifier is 'SUMMARY', summary file
is created;
%           'SUMMARY ONLY' - only summary is generated,
20 data for individual files are not saved
%
%    sfname - string. File name of a summary file
%    DEFAULT 'summary[date].dat'
%
25 % OUTPUT:
%
%    AnalyseDNA works on image files or montages. For
each image file it creates a tab-delimits file of
measured
30 %    parameters of all the objects in the montage with
the same base name as a montage file and extension
specified
```

```
%    by dext parameter (or .dat by default) and file
'errors[date].err' - with the list of files that matched
the
%    filemask but could not be processed.
5 %    If 'summary' or 'summary only' modifier is
specified, it also creates a single file.
'summary[date].dat' (or
%    different extension, if specified by DEXT) which
contains summary information for all analyzed files.
10 %
%    ALL OUTPUT FILES are saved in a directory specified
by OUTPATH parameter
%
%    RETURNS *files_analysed* - number of files that have
15 been successfully processed.
%
%    Column designations in the output files are
described in GetDNADData
%
20 % FILE NAME CONVENTIONS
%    AnalyseDNA attempts to identify a number for each
file to identify the file in summary output.
%    It does that by looking for the first space or
underscore, followed by a number and then takes
25 %    as many successive numbers as it can find. If it
fails to identify a number it assigns a
%    default which is -1
%
%
30 % SEE ALSO GetDNADData, GetSummaryData
%
% TO DO    improve error handling in opening and writing
files (GLOBAL error_file ?)
```

```
%           include procedures for writing text headers
into the output files

if nargin > 8
5   error ('Wrong number of input parameters');
end
if nargin > 1
    error ('Wrong number of output parameters: only one
allowed');
10 end

% set defaults
need_summary = 0;
summary_only = 0;
15 use_default_outpath = 0;
datestring = datestr(floor(now));
if nargin == 7      % set default summary file name
    sfname = ['summary' deblank(datestring)]; % extension
will be appended later based on dext
20   if deblank(upper(modifier)) == 'SUMMARY'
        need_summary = 1;
    elseif deblank(upper(modifier)) == 'SUMMARY ONLY'
        need_summary = 1;
        summary_only = 1;
25   else
        error (['Wrong parameter: unknown modifier '
modifier]);
    end
end

30
if nargin == 5
    % default data file extension
    set_dext = 'dat';
end
```



```
    if nargin == 4
        % default filter range
        filter_range = [];
    end
5   if nargin == 3
        ny = 1; % default number of images in montage along Y
    end
    if nargin == 2
        nx = 1;
10  end
    if nargin == 1
        use_default_outpath = 1;
    end
    if nargin == 0
15     filemask = '*.tif'
    end

    % check parameters
    if ( ~ischar(filemask) | ~ischar(dext) | ~ischar(sfname)
20  )
        error('Wrong parameter type: filename, filepath,
dext and sfname should be strings');
    end
    if ( ( size(nx) ~= [1 1] ) | ( size(ny) ~= [1 1] ) )
25     error('Wrong parameter type: nx and ny should be
scalars (1x1 arrays)');
    end
    if (~isempty(filter_range) & size(filter_range, 2) ~= 3)
        error('Wrong parameter type: filter range should be
30  [] or 3 - cols-wide array');
    end
    % end testing parameters

    % Generate list of files to process
```

```
datapath = getpath(filemask);
if use_default_outpath == 1
    outpath = datapath;
5  end
if exist(outpath, 'dir') ~= 7
    error(['Path ' outpath, 'not found. Exiting..']);
elseif exist(datapath, 'dir') ~= 7
    error(['Path ' datapath, 'not found. Exiting..']);
10 end

sfname = makefullname(outpath, sfname, dext);
if need_summary == 1
    if exist(sfname, 'file')
15         disp(['File ', sfname, 'already exists!']);
        input ('Press ^C to abort, Enter to delete and
continue');
        delete(sfname);
    end
20 end

flist = FileList(getfname(filemask), datapath);
numfiles = size(flist, 1); % total number of files to
25 process
disp(['About to process ', num2str(numfiles), ' files']);
%DEBUG - commented out "input" to run from Wrod
input('Press ^C to abort, Enter to continue');

30 % main loop where the job gets done:
error_file = makefullname(outpath, ['error' datestring
'.err']);
num_processed = 0;
num_error = 0;
```

```
for i = 1:numfiles
    % first generate file name for a data output file
    current_fullname = flist(i, :); % full name with path
    and extension
5    current_datafile = makefullname(outpath,
    makefname(getbasefname(current_fullname), dext) );

    %extract number from a filename
    fnumber = getfilenumber(current_fullname);
10

    % load an imagefile, record errors
    read_error = 0;
    try
        I = imread(current_fullname);
15        %DEBUG
        disp(['Image file #', num2str(fnumber), '
loaded']);
    catch
        % record file-opening error in an error_file
20        read_error = 1;
        num_error = num_error +1;
        msg = [current_fullname ': ' lasterr];
        add_error_msg(error_file, msg);
    end
25

    % extract and write data to a file in outpath
    if read_error ~=1
        if (need_summary == 0)
            %DEBUG
30            disp(['Starting analysis of file #',
num2str(fnumber), '.']);
            current_data = GetDNADData(I, nx, ny, fnumber);
            %DEBUG
```

```

        disp(['Finished analysis of file #',
num2str(fnumber), '.']);
        %load current_data.mat 'current_data';
        write_data(current_data, current_datafile);
5         else      %summary needed
            %DEBUG
            [current_data, current_summary] = GetDNADData(I,
nx, ny, fnumber, filter_range);
            %load current_data.mat 'current_data';
10         %load current_summary.mat 'current_summary';
            write_summary (current_summary, sfname);
            if summary_only ~= 1
                write_data(current_data, current_datafile);
            end
15         end
        end
    end % of the main for loop
    num_processed = numfiles - num_error;

20  %=====end function AnalyseDNA()
    %=====

    %=====
    %=====

25  function result = add_error_msg(filename, msg)
    % adds string MSG to an errorfile FILENAME
    % returns 1 if success, 0 if failure

    err_FID = fopen(filename, 'at');
30  if err_FID == -1
        warning(['Can not open error file ' filename]);
    else
        fprintf(err_FID, '%s\n', msg);
        fclose(err_FID);

```

```

end
%=====end function add_error_masg()
=====

5  %=====
=====
function N = getfilenumber(fname)
% returns the first number extracted from a file name
% (string) or -1 if fails to extract any number
10 numbers = NumbersFromString( getfname(fname) ); % vector
    of all numbers encoded in the name

                                % (but not in the path, even if
present)
15 if isempty(numbers)
    N = (-1); % return -1 if no numbers found in the
name
else
    N = numbers(1);
20 end

%===== end function getfilenumber()
=====

25 %=====
=====
function result = write_data(data_array, file_name)
% writes data in a data_array in a tab-delimited ascii
file.
30 % result is 0 if success and -1 if failure
% if file_name exists, overwrites it
result = -1;
try
    fid = fopen(file_name, 'wt');

```

```

        if fid ~= -1
            for k = 1:size(data_array, 1)
                fprintf(fid, '%g\t', data_array(k, :));
                fprintf (fid, '\n');
5         end
            test = fclose(fid);
            result = -1;
        catch
            result = -1;
10    end

%===== end function write_data()
=====

15 %=====
=====

function result = write_summary (s_vector, file_name)
% appends summary vector s_vector to a file_name (ASCII
tab-delimited file).
20 % if file_name does not exist, creates it.
% result is 0 if success and -1 if failure
%
result = -1;
try
25    % debug
        fid = fopen(file_name, 'at');
        result = fprintf(fid, '%g\t', s_vector);
        result = fprintf(fid, '\n');
        result = fclose(fid);
30    result = 0;
    catch
        result = -1;
    end

```

```

% ===== end function write_summary()
=====

function Data = GetObjectsData(I, Ilabel)
5 % GetObjectsData returns array measurements of objects in
image "I" masked by "Ilabel"
% EV 2-3-99; 2-10-99
% OData = GetObjectsData(I, Ilabel) returns an array of
morphological and intensity measurements
10 % taken from a grayscale image "I". Objects are
identified on a mask image Ilabel, usually
% created by bwlabel()
% OUTPUT:
% Each row in the output array OData represents
15 individual object
% columns contain the following measurements:
%
% 1 - Index ("number" of an object); 8 -
Solidity;
20 % 2 - X coordinate of the center of mass; 9 - Extent;
% 3 - Y coordinate -"- ; 10 - Total
Intensity;
% 4 - Total Area (in pixels); 11 - Avg.
Intensity;
25 % 5 - Ratio of MajorAxis/MinorAxis; 12 - Median
Intensity;
% 6 - Eccentricity; 13 - Intensity of
20% bright pixel
% 7 - EquivDiameter; 14 - Intensity of
30 80% bright pixel
%
% For details on morphological parameters see information
on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.
% Procedures in this file are documented in notebook file
"MATLAB Measuring Nuclei (1) 1-29-98.doc"

5
if (nargin ~= 2)
    error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
10    error ('function has 1 output argument (array X by
    14)');
end

% finished checking arguments

15
% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
'MajorAxisLength',...
20    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
    'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
25 intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
30 %now convert ImStats into array and add intensity data to
it
for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
```



```

        OData(k, 3) = ImStats(k).Centroid(2);
        OData(k, 4) = ImStats(k).Area;
        OData(k, 5) = (ImStats(k).MajorAxisLength) /
        (ImStats(k).MinorAxisLength);
5         OData(k, 6) = ImStats(k).Eccentricity ;
        OData(k, 7) = ImStats(k).EquivDiameter;
        OData(k, 8) = ImStats(k).Solidity;
        OData(k, 9) = ImStats(k).Extent;

10         % now collect and assign intensity parameters from
        image I

        object_pixels = find( Ilabel == k);
        object_area = size(object_pixels, 1); %same as total
15 number of pixels in the object
        object_intensities = double(I(object_pixels)); %
        need to convert to double to do math
        sorted_intensities = sort(object_intensities); %
        will need to get median, 20% and 80% pixels
20         total_intensity = sum(object_intensities, 1);
        avg_intensity = total_intensity / object_area;
        median_intensity = sorted_intensities( floor(
        object_area/2 ) + 1 );
        pix20 = sorted_intensities( floor(object_area*0.2)+1
25 ) ; %brightest pixel among dimmest 20%
        pix80 = sorted_intensities( floor(object_area*0.8)+1
        ) ;

        OData(k, 10) = total_intensity;
30         OData(k, 11) = avg_intensity;
        OData(k, 12) = median_intensity;
        OData(k, 13) = pix20; %brightest pixel among dimmest
        20%

```

```

        OData(k, 14) = pix80; %dimmet pixel among brightest
    20%
    end %for

5  %===== end function
    GetObjectsData()=====

    function Imask = MaskDNA1(I);
10  % MaskDNA1 - generates binary mask for cell nuclei
    through edge detection
    % EV 1-22-99; 2-6-99; 2-10-99
    % Imask = MaskDNA1(I)
    % PARAMETERS
15  % I - intensity image (grayscale)
    % OUTPUT
    % Imask - BW image with objects from I
    %
    % For more details see Notebook Matlab_DNA_masking1_1-22-
20  99.doc
    % Uses SDC Morphology Toolbox V0.7

    if (nargin ~= 1)
        error('Wrong number of input parameters');
25  end
    if (nargout ~= 1)
        error('Wrong number of output parameters: one output
        argument should be provided');
    end
30

    Imask = edge(I, 'canny');
    Imask = mm dil(Imask, mmsecross(1));
    Imask = mmero ( mmclose(Imask,mmsecross(1)));

```

```

Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
5  Imask = medfilt2(Imask, [5 5]);

%=====end MaskDNA1 =====

```

10        Given the list of image files or montages of images as an input, this program creates an individual file for each image that contains the following quantitative measurements for all objects identified in the image:

1 - Index ("number" of an object);	8 - Solidity;
2 - X coordinate of the center of mass;	9 - Extent;
15 3 - Y coordinate        "-";	10 - Total Intensity;
4 - Total Area (in pixels);	11 - Avg. Intensity;
5 - Ratio of MajorAxis/MinorAxis;	12 - Median Intensity;
6 - Eccentricity;	13 - Intensity of 20% bright pixel
7 - EquivDiameter;	14 - Intensity of 80% bright pixel

20        A fragment of an output for a single file, containing 9 images of cells stained for DNA and acquired with a 10x objective. A montage image that was used as a source to generate data in A is presented in Fig. 17.

25        The same program also summarizes measurements across many files and performs statistical analysis of the summary data. It creates a summary file with the following data:

1 - Image file number;	
2 - Average object Area (in pixels);	3 - STD (standard deviation) of
2;	
30 4 - Avg. of Ratio of MajorAxis/MinorAxis;	5 - STD of 4;
6 - Avg. Eccentricity;	7 - STD of 6;
8 - Avg. EquivDiameter;	9 - STD of 8;
10 - Avg. of Solidity;	11 - STD of 10;

12 - Avg. of Extent;	13 - STD of 11
14 - Avg. of objects Total Intensity;	15 - STD of 14
16 - Avg. of objects Avg Intensity;	16 - STD of 15
18 - Avg. of objects Median intensity;	19 - STD of 18
20 - Avg. of objects intensity of 20% bright pixel;	21 - STD of 19
22 - Avg. of objects intensity of 80% bright pixel;	23 - STD of 21

An example of summary output obtained by running AnalyseDNA against 10 montage files also is shown in Appendix B.

10           A seventh experiment was conducted in order to use sequence analysis algorithms to analyze features of cell images. In this experiment, HeLa cells were treated for 24 hours with several different compounds, and then fixed, and stained with a fluorescent DNA dye. One image of these cells was acquired for each of the treatments and morphometric parameters and features were measured:

15           Resulting measurements were arranged into a string of numbers and reduced to a pseudo- nucleic acid sequence using following rules: At any given position in the sequence a number was substituted by "t" (a code for thymidine) if its value is among highest 25% of the values at the corresponding position in the data set, "g" if it is between 50% and 25%, "c" if it is between 75% and 50%, and "a" if it  
20 belongs to lowest 25% of values. Thus one descriptor or sequence was generated per treatment as illustrated in Fig. 18.

Resulting sequences were clustered using an AlignX module commercial software package Vector NTI (<http://informaxinc.com>), which uses a Neighbor Joining algorithm for sequence clustering.

25           The resulting dendrogram is presented in Fig 18. On the dendrogram the closest "leafs" correspond to the closest pseudo-sequences. Interestingly, compounds with similar mechanisms of action cluster together on the dendrogram. Another example of the generation of pseudo-sequences and clustering is shown in Fig. 19.

30           In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs such as clinical trial and patient response information will be used in a similar fashion as the pre-clinical

information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions can be able to provide predictive information for this aspect of drug development.

Although the above has generally described the present invention  
5 according to specific systems, the present invention has a much broader range of applicability. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many  
10 different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives.

## APPENDIX A

EV Table 1.doc

Example of the output of AnalyseDNA.m program  
(measurements for a single 3 by 3 montage image)

File	Subimage	object	X coord	Y coord	Area	Area ratio	Centrality	Equilibrium	Solidity	Extant	Intensity	Avg. Intensity	Median Intensity	20% pth.	80% pth.
1	1	1	12,1891	152,625	145	1.17221	0.322434	13.5815	0.933567	0.739796	4803	31.7586	31	23	37
1	2	1	14,352	416,022	125	1.03194	0.742471	12.4157	0.905791	0.781735	4606	35.816	38	30	45
1	3	1	20,1073	73,8079	177	1.09145	0.412785	15.0171	0.911028	0.431406	1769	76.7925	81	22	31
1	4	1	21,4186	402,744	43	1.38215	0.438004	7.39928	0.910894	0.471837	3690	85.816	87	47	105
1	5	1	27,0928	181	96	1.04889	0.445194	11.0538	0.888889	0.471329	4502	16.9358	49	38	56
1	6	1	30,2132	255,334	206	2.23106	0.303209	16.1953	0.977928	0.715778	6360	30.9709	23	21	37
1	7	1	32,4619	161,573	89	1.31984	0.714656	10.4451	0.970083	0.711667	4725	47.4719	50	39	56
1	8	1	35,0411	14,9726	146	1.25176	0.701495	13.4317	0.979936	0.718978	5815	37.0819	40	29	44
1	9	1	37,746	266,021	67	1.41642	0.435412	7.35576	0.87037	0.452718	4667	141.853	142	113	171
1	10	1	49,1078	170,001	232	1.70491	0.451127	17.1187	0.832941	0.707023	9832	42.2783	45	33	51
1	11	1	56,0749	125,534	221	1.35704	0.455555	14.7716	0.974686	0.466235	7010	31.8532	33	25	33
1	12	1	57,7755	44,9922	143	1.31477	0.463201	13.4609	0.907407	0.706731	4765	32.415	31	26	38
1	13	1	57,7755	246,851	171	2.72725	0.491953	14.7555	0.821418	0.704612	4765	34.8121	36	43	68
1	14	1	58,4028	282,272	208	1.72782	0.454914	16.1953	0.931161	0.432135	7137	31.4166	37	28	43
1	15	1	68,1716	333,181	315	1.11134	0.432466	20.0167	0.735	0.582556	15151	46.0984	50	31	51
1	16	1	68,1716	333,181	315	1.70167	0.409064	16.7346	0.87092	0.447059	9809	44.5844	46	35	54
1	17	1	65,1109	602,411	220	1.71588	0.412422	12.3142	0.911111	0.473529	6324	39.3577	41	30	47
1	18	1	71,4619	643,13	185	1.71588	0.412422	12.3142	0.911111	0.473529	6324	39.3577	41	30	47
1	19	1	73,624	181,054	125	1.6379	0.491953	19.7368	0.87092	0.447059	9809	44.5844	46	35	54
1	20	1	77,4868	321,313	206	1.3357	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	21	1	78,7377	208,27	172	1.43713	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	22	1	81,4786	55,5912	117	1.43713	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	23	1	88,7292	281,534	373	2.13388	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	24	1	86,1765	341,576	85	1.02573	0.717365	13.4935	0.910789	0.718444	4878	31.1117	35	27	41
1	25	1	88,1408	176,231	163	1.34533	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	26	1	91,4529	316,924	170	1.97353	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	27	1	97,7604	317,795	286	1.97353	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	28	1	96,9451	240,462	119	1.2774	0.422219	12.3533	0.921875	0.766224	4872	60.377	43	33	51
1	29	1	96,9451	240,462	119	1.2774	0.422219	12.3533	0.921875	0.766224	4872	60.377	43	33	51
1	30	1	101,033	92,3279	122	1.48115	0.730977	12.4634	0.915736	0.766224	4872	38.2213	40	31	47
1	31	1	101,033	155,307	134	1.5208	0.730977	12.4634	0.915736	0.766224	4872	38.2213	40	31	47
1	32	1	105,356	97,1271	118	1.70329	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	33	1	121,31	285,08	326	1.70329	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	34	1	125,322	170,645	141	1.51045	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	35	1	128,98	60,3355	132	1.75429	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	36	1	137,082	242,354	166	1.445	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	37	1	130,903	411,5	164	1.18276	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	38	1	132,139	392,545	197	1.18276	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	39	1	132,139	392,545	197	1.18276	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	40	1	131,511	201,028	101	1.20213	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	41	1	131,511	201,028	101	1.20213	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	42	1	140,093	101,008	121	1.47487	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	43	1	141,061	59,8159	272	1.35319	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	44	1	141,061	59,8159	272	1.35319	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	45	1	151,66	256,921	276	1.08068	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	46	1	155,688	178,546	141	1.31335	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	47	1	160,075	242,354	166	1.71551	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	48	1	166,028	11,7677	198	1.42126	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	49	1	169,613	136,767	217	1.74718	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	50	1	176,014	356,115	222	1.41188	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	51	1	175,788	192,983	119	1.12784	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	52	1	177,181	210,814	127	1.14539	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	53	1	176,367	610,524	147	1.24127	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	54	1	182,1	392,576	170	1.37768	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	55	1	185,780	242,354	196	1.17904	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	56	1	200,742	32,7118	213	1.34983	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	57	1	199,358	156,725	51	1.01623	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	58	1	201,47	185,871	244	1.34013	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	59	1	201,752	70,0135	220	1.49329	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	60	1	212,384	346,453	197	1.13759	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	61	1	220,856	30,6237	154	1.47487	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	62	1	216,360	234,29	183	1.34189	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	63	1	216,292	293,953	173	1.41627	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	64	1	211,331	330,721	172	1.72537	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	65	1	211,380	627,1	204	1.79372	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	66	1	222,326	151,769	121	1.2181	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	67	1	219,071	488,948	435	1.20117	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47
1	68	1	230,244	245,186	167	1.18408	0.403561	12.4634	0.90468	0.473529	6324	39.3577	41	30	47

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1	261.509	88.7837	224	1.47991	0.816732	16.808	0.419594	0.434341	8827	39.4063	42	39
2	268.031	222.144	340	1.151661	0.430019	11.232	0.31954	0.716555	5015	31.0013	33	38
3	270.234	413.024	37	1.151661	0.430019	9.30149	0.415	0.7	4332	56.5195	39	41
4	276.945	434.016	163	1.50893	0.777002	11.4042	0.51573	0.716555	1720	71.9571	30	36
5	281.061	398.818	66	1.031149	0.245208	9.167	0.316463	0.610015	1410	67.2327	71	80
6	283.602	375.25	231	1.95991	0.860038	17.8769	0.868926	0.287619	10500	61.8327	13	32
7	286.293	233.801	151	1.45064	0.295632	11.3175	0.316973	0.480031	5126	31.9006	33	35
8	288.933	209.802	111	1.31512	0.69672	11.8882	0.917355	0.720719	4928	41.7658	35	36
9	291.624	248.218	131	1.58185	0.714929	12.9119	0.907322	0.482292	9423	33.5144	71	56
10	294.315	171.24	204	2.05413	0.873163	16.1165	0.918919	0.396051	7031	31.5637	35	38
11	297.006	235.094	247	1.27832	0.622935	15.118	0.81231	0.745506	9202	41.1167	63	45
12	300.697	371.32	130	1.10212	0.422329	12.8194	0.920215	0.817308	4387	31.1114	55	41
13	303.388	291.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
14	306.079	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
15	308.770	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
16	311.461	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
17	314.152	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
18	316.843	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
19	319.534	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
20	322.225	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
21	324.916	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
22	327.607	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
23	330.298	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
24	332.989	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
25	335.680	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
26	338.371	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
27	341.062	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
28	343.753	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
29	346.444	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
30	349.135	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
31	351.826	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
32	354.517	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
33	357.208	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
34	359.899	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
35	362.590	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
36	365.281	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
37	367.972	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
38	370.663	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
39	373.354	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
40	376.045	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
41	378.736	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
42	381.427	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
43	384.118	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
44	386.809	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
45	389.500	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
46	392.191	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
47	394.882	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
48	397.573	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
49	400.264	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
50	402.955	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
51	405.646	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
52	408.337	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
53	411.028	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
54	413.719	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
55	416.410	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
56	419.101	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
57	421.792	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
58	424.483	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
59	427.174	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
60	429.865	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
61	432.556	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
62	435.247	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
63	437.938	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
64	440.629	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
65	443.320	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
66	446.011	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
67	448.702	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
68	451.393	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
69	454.084	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
70	456.775	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
71	459.466	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
72	462.157	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
73	464.848	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
74	467.539	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
75	470.230	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
76	472.921	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
77	475.612	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
78	478.303	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
79	480.994	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
80	483.685	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
81	486.376	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
82	489.067	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
83	491.758	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
84	494.449	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
85	497.140	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
86	500.831	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387	41.1167	63	45
87	503.522	231.118	85	1.47318	0.801747	10.4031	0.923913	0.817308	4387</			

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1	136	445.48	366.449	239	2.40072	0.321085	19.5115	0.49521	0.64891	14065	47.0101	50	38	37
2	137	449.732	366.241	190	1.35179	0.321058	15.5536	0.495056	0.646667	8786	46.2421	50	35	37
3	138	452.238	377.287	195	1.36728	0.379285	15.7357	0.532014	0.72181	5408	27.7323	29	23	31
4	139	454.088	385.078	102	1.21521	0.364019	11.3161	0.318319	0.713787	5508	54	38	35	40
5	140	455.517	391.565	147	1.43922	0.450132	13.4609	0.380216	0.718471	5099	34.4871	37	25	41
6	141	456.745	396.361	176	1.49474	0.744034	14.5956	0.521116	0.670559	8466	55.0528	40	45	48
7	142	457.811	400.451	176	1.20928	0.562286	10.3179	0.48172	0.483232	4200	32.9024	35	45	55
8	143	458.745	404.451	82	1.49712	0.741206	16.3907	0.505579	0.702322	5218	35.7028	41	35	55
9	144	459.571	407.451	211	1.16551	0.513457	7.0668	0.480931	0.747475	4079	35.7216	56	44	66
10	145	460.301	410.451	76	1.44756	0.722029	9.7302	0.473118	0.6597	3383	37.7216	59	48	70
11	146	460.931	413.451	69	1.44756	0.722029	9.7302	0.473118	0.6597	3383	37.7216	59	48	70
12	147	461.561	416.451	237	2.55212	0.320687	19.4161	0.339733	0.53302	11784	39.4748	41	31	48
13	148	462.191	419.451	155	1.43041	0.761975	14.0182	0.339733	0.531964	4887	39.4748	41	31	48
14	149	462.821	422.451	233	1.38716	0.454216	11.224	0.339733	0.531964	4887	39.4748	41	31	48
15	150	463.451	425.451	191	1.47447	0.40209	15.5945	0.339733	0.531964	4887	39.4748	41	31	48
16	151	464.081	428.451	195	1.23958	0.561865	16.4985	0.339733	0.531964	4887	39.4748	41	31	48
17	152	464.711	431.451	215	2.19727	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
18	153	465.341	434.451	176	1.49502	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
19	154	465.971	437.451	176	1.20361	0.44153	14.5446	0.339733	0.531964	4887	39.4748	41	31	48
20	155	466.601	440.451	193	1.37009	0.44153	14.5446	0.339733	0.531964	4887	39.4748	41	31	48
21	156	467.231	443.451	193	1.37009	0.44153	14.5446	0.339733	0.531964	4887	39.4748	41	31	48
22	157	467.861	446.451	210	1.44688	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
23	158	468.491	449.451	141	0.23467	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
24	159	469.121	452.451	124	1.39515	0.497472	12.5651	0.339733	0.531964	4887	39.4748	41	31	48
25	160	469.751	455.451	164	1.39515	0.497472	12.5651	0.339733	0.531964	4887	39.4748	41	31	48
26	161	470.381	458.451	174	1.55706	0.764515	16.4503	0.339733	0.531964	4887	39.4748	41	31	48
27	162	471.011	461.451	174	1.47033	0.400586	16.4843	0.339733	0.531964	4887	39.4748	41	31	48
28	163	471.641	464.451	22	1.38172	0.771787	15.29257	0.339733	0.531964	4887	39.4748	41	31	48
29	164	472.271	467.451	63	1.48078	0.444693	15.29257	0.339733	0.531964	4887	39.4748	41	31	48
30	165	472.901	470.451	188	1.16558	0.444693	15.29257	0.339733	0.531964	4887	39.4748	41	31	48
31	166	473.531	473.451	188	2.25576	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
32	167	474.161	476.451	109	1.36139	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
33	168	474.791	479.451	109	1.36139	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
34	169	475.421	482.451	105	1.46152	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
35	170	476.051	485.451	105	1.46152	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
36	171	476.681	488.451	192	1.46152	0.400435	16.4682	0.339733	0.531964	4887	39.4748	41	31	48
37	172	477.311	491.451	252	1.52487	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
38	173	477.941	494.451	130	1.2305	0.36271	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
39	174	478.571	497.451	128	1.2305	0.36271	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
40	175	479.201	500.451	165	1.2113	0.364315	15.7662	0.339733	0.531964	4887	39.4748	41	31	48
41	176	479.831	503.451	165	1.53776	0.759591	15.7662	0.339733	0.531964	4887	39.4748	41	31	48
42	177	480.461	506.451	104	1.34258	0.44153	14.5446	0.339733	0.531964	4887	39.4748	41	31	48
43	178	481.091	509.451	160	2.13828	0.44153	14.5446	0.339733	0.531964	4887	39.4748	41	31	48
44	179	481.721	512.451	123	1.14789	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
45	180	482.351	515.451	114	1.41038	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
46	181	482.981	518.451	114	1.7702	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
47	182	483.611	521.451	216	1.76228	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
48	183	484.241	524.451	216	1.76228	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
49	184	484.871	527.451	114	1.76228	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
50	185	485.501	530.451	114	1.76228	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
51	186	486.131	533.451	102	1.2221	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
52	187	486.761	536.451	102	1.2221	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
53	188	487.391	539.451	102	1.2221	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
54	189	488.021	542.451	108	1.76228	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
55	190	488.651	545.451	254	2.28656	0.494543	16.5318	0.339733	0.531964	4887	39.4748	41	31	48
56	191	489.281	548.451	201	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
57	192	489.911	551.451	201	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
58	193	490.541	554.451	201	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
59	194	491.171	557.451	201	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
60	195	491.801	560.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
61	196	492.431	563.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
62	197	493.061	566.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
63	198	493.691	569.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
64	199	494.321	572.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
65	200	494.951	575.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
66	201	495.581	578.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
67	202	496.211	581.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
68	203	496.841	584.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
69	204	497.471	587.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
70	205	498.101	590.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
71	206	498.731	593.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
72	207	499.361	596.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
73	208	499.991	599.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
74	209	500.621	602.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
75	210	501.251	605.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
76	211	501.881	608.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
77	212	502.511	611.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
78	213	503.141	614.451	267	1.44383	0.735426	17.9125	0.339733	0.531964	4887	39.4748	41	31	48
79	214	503.771	617.45											

EV Table I.doc
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EV Table 1.doc

1	150	491.466	286.065	35	1.14938	0.341335	6.47558	0.945916	0.433333	3545	101.261	105	88	117
2	151	497.438	76.3769	130	2.04206	0.871091	12.4655	0.902778	0.637255	4533	35.0231	36	26	43
3	152	499.434	144.308	128	1.24075	0.531946	12.7662	0.920865	0.811111	4856	31.9375	39	30	46
4	153	501.434	285.462	39	1.4304	0.713017	7.0673	0.906577	0.911111	3600	31.3359	36	29	45
5	154	503.434	426.616	127	1.3561	0.654419	13.3716	0.922212	0.710769	4832	31.7895	35	28	44
6	155	505.434	567.770	126	1.2818	0.595926	14.7861	0.938745	0.752963	5190	31.7895	30	27	43
7	156	507.434	708.924	125	1.2075	0.537433	16.2013	0.955073	0.795311	5548	31.7895	25	26	42
8	157	509.434	850.078	124	1.1332	0.479940	17.6165	0.971306	0.837653	5906	31.7895	20	25	41
9	158	511.434	991.232	123	1.0589	0.422447	19.0317	0.987539	0.879995	6264	31.7895	15	24	40
10	159	513.434	1132.386	122	0.9846	0.364954	20.4469	1.003772	0.922333	6622	31.7895	10	23	39
11	160	515.434	1273.540	121	0.9103	0.307461	21.8621	1.019995	0.964667	6980	31.7895	5	22	38
12	161	517.434	1414.694	120	0.8360	0.250000	23.2773	1.036218	1.006000	7338	31.7895	0	21	37
13	162	519.434	1555.848	119	0.7617	0.192500	24.6925	1.052441	1.047333	7696	31.7895	0	20	36
14	163	521.434	1696.999	118	0.6874	0.135000	26.1077	1.068664	1.088667	8054	31.7895	0	19	35
15	164	523.434	1838.153	117	0.6131	0.077500	27.5229	1.084887	1.129995	8412	31.7895	0	18	34
16	165	525.434	1979.307	116	0.5388	0.020000	28.9381	1.101110	1.171333	8770	31.7895	0	17	33
17	166	527.434	2120.461	115	0.4645	0.000000	30.3533	1.117333	1.212667	9128	31.7895	0	16	32
18	167	529.434	2261.615	114	0.3902	0.000000	31.7685	1.133556	1.254000	9486	31.7895	0	15	31
19	168	531.434	2402.769	113	0.3159	0.000000	33.1837	1.149779	1.295333	9844	31.7895	0	14	30
20	169	533.434	2543.923	112	0.2416	0.000000	34.5989	1.166002	1.336667	10202	31.7895	0	13	29
21	170	535.434	2685.077	111	0.1673	0.000000	36.0141	1.182225	1.378000	10560	31.7895	0	12	28
22	171	537.434	2826.231	110	0.0930	0.000000	37.4293	1.198448	1.419333	10918	31.7895	0	11	27
23	172	539.434	2967.385	109	0.0187	0.000000	38.8445	1.214671	1.460667	11276	31.7895	0	10	26
24	173	541.434	3108.539	108	0.0000	0.000000	40.2597	1.230894	1.502000	11634	31.7895	0	9	25
25	174	543.434	3249.693	107	0.0000	0.000000	41.6749	1.247117	1.543333	11992	31.7895	0	8	24
26	175	545.434	3390.847	106	0.0000	0.000000	43.0901	1.263340	1.584667	12350	31.7895	0	7	23
27	176	547.434	3531.999	105	0.0000	0.000000	44.5053	1.279563	1.626000	12708	31.7895	0	6	22
28	177	549.434	3673.153	104	0.0000	0.000000	45.9205	1.295786	1.667333	13066	31.7895	0	5	21
29	178	551.434	3814.307	103	0.0000	0.000000	47.3357	1.312009	1.708667	13424	31.7895	0	4	20
30	179	553.434	3955.461	102	0.0000	0.000000	48.7509	1.328232	1.750000	13782	31.7895	0	3	19
31	180	555.434	4096.615	101	0.0000	0.000000	50.1661	1.344455	1.791333	14140	31.7895	0	2	18
32	181	557.434	4237.769	100	0.0000	0.000000	51.5813	1.360678	1.832667	14498	31.7895	0	1	17
33	182	559.434	4378.923	99	0.0000	0.000000	53.0000	1.376901	1.874000	14856	31.7895	0	0	16
34	183	561.434	4519.077	98	0.0000	0.000000	54.4152	1.393124	1.915333	15214	31.7895	0	0	15
35	184	563.434	4660.231	97	0.0000	0.000000	55.8304	1.409347	1.956667	15572	31.7895	0	0	14
36	185	565.434	4801.385	96	0.0000	0.000000	57.2456	1.425570	1.998000	15930	31.7895	0	0	13
37	186	567.434	4942.539	95	0.0000	0.000000	58.6608	1.441793	2.039333	16288	31.7895	0	0	12
38	187	569.434	5083.693	94	0.0000	0.000000	60.0760	1.458016	2.080667	16646	31.7895	0	0	11
39	188	571.434	5224.847	93	0.0000	0.000000	61.4912	1.474239	2.122000	17004	31.7895	0	0	10
40	189	573.434	5365.999	92	0.0000	0.000000	62.9064	1.490462	2.163333	17362	31.7895	0	0	9
41	190	575.434	5507.153	91	0.0000	0.000000	64.3216	1.506685	2.204667	17720	31.7895	0	0	8
42	191	577.434	5648.307	90	0.0000	0.000000	65.7368	1.522908	2.246000	18078	31.7895	0	0	7
43	192	579.434	5789.461	89	0.0000	0.000000	67.1520	1.539131	2.287333	18436	31.7895	0	0	6
44	193	581.434	5930.615	88	0.0000	0.000000	68.5672	1.555354	2.328667	18794	31.7895	0	0	5
45	194	583.434	6071.769	87	0.0000	0.000000	69.9824	1.571577	2.370000	19152	31.7895	0	0	4
46	195	585.434	6212.923	86	0.0000	0.000000	71.3976	1.587800	2.411333	19510	31.7895	0	0	3
47	196	587.434	6354.077	85	0.0000	0.000000	72.8128	1.604023	2.452667	19868	31.7895	0	0	2
48	197	589.434	6495.231	84	0.0000	0.000000	74.2280	1.620246	2.494000	20226	31.7895	0	0	1
49	198	591.434	6636.385	83	0.0000	0.000000	75.6432	1.636469	2.535333	20584	31.7895	0	0	0
50	199	593.434	6777.539	82	0.0000	0.000000	77.0584	1.652692	2.576667	20942	31.7895	0	0	0
51	200	595.434	6918.693	81	0.0000	0.000000	78.4736	1.668915	2.618000	21300	31.7895	0	0	0
52	201	597.434	7059.847	80	0.0000	0.000000	79.8888	1.685138	2.659333	21658	31.7895	0	0	0
53	202	599.434	7200.999	79	0.0000	0.000000	81.3040	1.701361	2.700667	22016	31.7895	0	0	0
54	203	601.434	7342.153	78	0.0000	0.000000	82.7192	1.717584	2.742000	22374	31.7895	0	0	0
55	204	603.434	7483.307	77	0.0000	0.000000	84.1344	1.733807	2.783333	22732	31.7895	0	0	0
56	205	605.434	7624.461	76	0.0000	0.000000	85.5496	1.750030	2.824667	23090	31.7895	0	0	0
57	206	607.434	7765.615	75	0.0000	0.000000	86.9648	1.766253	2.866000	23448	31.7895	0	0	0
58	207	609.434	7906.769	74	0.0000	0.000000	88.3800	1.782476	2.907333	23806	31.7895	0	0	0
59	208	611.434	8047.923	73	0.0000	0.000000	89.7952	1.798699	2.948667	24164	31.7895	0	0	0
60	209	613.434	8189.077	72	0.0000	0.000000	91.2104	1.814922	2.990000	24522	31.7895	0	0	0
61	210	615.434	8330.231	71	0.0000	0.000000	92.6256	1.831145	3.031333	24880	31.7895	0	0	0
62	211	617.434	8471.385	70	0.0000	0.000000	94.0408	1.847368	3.072667	25238	31.7895	0	0	0
63	212	619.434	8612.539	69	0.0000	0.000000	95.4560	1.863591	3.114000	25596	31.7895	0	0	0
64	213	621.434	8753.693	68	0.0000	0.000000	96.8712	1.879814	3.155333	25954	31.7895	0	0	0
65	214	623.434	8894.847	67	0.0000	0.000000	98.2864	1.896037	3.196667	26312	31.7895	0	0	0
66	215	625.434	9035.999	66	0.0000	0.000000	99.7016	1.912260	3.238000	26670	31.7895	0	0	0
67	216	627.434	9177.153	65	0.0000	0.000000	101.1168	1.928483	3.279333	27028	31.7895	0	0	0
68	217	629.434	9318.307	64	0.0000	0.000000	102.5320	1.944706	3.320667	27386	31.7895	0	0	0
69	218	631.434	9459.461	63	0.0000	0.000000	103.9472	1.960929	3.362000	27744	31.7895	0	0	0
70	219	633.434	9600.615	62	0.0000	0.000000	105.3624	1.977152	3.403333	28102	31.7895	0	0	0
71	220	635.434	9741.769	61	0.0000	0.000000	106.7776	1.993375	3.444667	28460	31.7895	0	0	0
72	221	637.434	9882.923	60	0.0000	0.000000	108.1928	2.009598	3.486000	28818	31.7895	0	0	0
73	222	639.434	10024.077	59	0.0000	0.000000	109.6080	2.025821	3.527333	29176	31.7895	0	0	0
74	223	641.434	10165.231	58	0.0000	0.000000	111.0232	2.042044	3.568667	29534	31.7895	0	0	0
75	224	643.434	10306.385	57	0.0000	0.000000	112.4384	2.058267	3.610000	29892	31.7895	0	0	0
76	225	645.434	10447.539	56	0.0000	0.000000	113.8536	2.074490	3.651333	30250	31.7895	0	0	0
77	226	647.434	10588.693	55	0.0000	0.000000	115.2688	2.090713	3.692667	30608	31.7895	0	0	0
78	227	649.434	10729.847	54	0.0000	0.000000	116.6840	2.106936	3.734000	30966	31.7895	0	0	0
79	228	651.434	10870.999	53	0.0000	0.000000	118.0992	2.123159	3.775333	31324	31.7895	0		

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EV Table 1.doc	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35		
	216.483	66.3186	324	1.70565	0.110106	20.4218	0.085812	0.703158	15684	16.588	49	38	25	35	39	35	39	35	39	35	39	35	39	35	39	35	39	35	39	35	39	35	39	35	39	35	39
	216.451	115.39	145	1.57559	0.727271	12.9815	0.917732	0.637115	4989	53.771	56	41	33	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
	215.452	241.232	172	1.4535	0.727314	16.8615	0.905759	0.664509	5192	53.771	56	41	33	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
	215.465	291.404	146	1.74553	0.816927	12.7213	0.905759	0.618683	4158	61.6081	44	31	24	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	265.892	35.5	146	1.69533	0.809014	19.1492	0.925373	0.635033	7114	61.6731	44	31	24	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	267.411	474.44	488	1.69274	0.809253	15.9115	0.91018	0.703708	12451	61.8236	45	31	24	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
	276.032	256.171	152	1.60284	0.821418	12.4143	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	288.874	481.764	123	1.8018	0.821418	12.4143	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	295.075	627.752	166	1.89538	0.821418	12.4143	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	300.661	175.511	122	1.79288	0.820231	12.4531	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	318.106	113.218	134	1.95255	0.825216	12.7311	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	316.432	110.751	131	1.95255	0.825216	12.7311	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
	326.411	418.425	193	1.64851	0.732210	12.4235	0.917293	0.727374	5246	61.7015	36	28	23	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20									

EV	Table	1.docx
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EV Table 1.duc

1	106	359.018	312.36	114	1.78044	0.827612	12.0418	0.497638	0.676376	6181	36.6751	38	15
2	107	361.714	169.031	129	1.40135	0.781018	12.8159	0.508451	0.661538	7535	58.4186	46	71
3	108	370.42	126.784	132	1.21766	0.64347	11.9416	0.503274	0.717848	6218	37.6007	30	66
4	109	379.666	101.258	97	1.21577	0.553737	11.1321	0.498118	0.731810	6114	42.7716	44	34
5	110	382.327	604.184	182	1.14028	0.420255	15.4204	0.512195	0.411111	6510	16.1497	36	57
6	111	386.96	392.188	203	1.42054	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
7	112	397.155	237.227	174	1.35594	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
8	113	398.666	241.441	174	1.42054	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
9	114	401.428	207.472	114	1.51997	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
10	115	395.317	272.314	114	2.04919	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
11	116	414.493	272.019	384	1.50902	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
12	117	421.434	70.7842	159	1.42116	0.421935	19.4416	0.513199	0.43125	61210	34.9167	39	61
13	118	429	14	14	1.78044	0.827612	12.0418	0.497638	0.676376	6181	36.6751	38	15
14	119	438.508	178.395	132	1.28321	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
15	120	442.416	171.2	395	1.48193	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
16	121	435.618	108.709	91	1.42734	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
17	122	435.959	245.441	131	1.74057	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
18	123	441.345	472.391	110	1.52612	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
19	124	447.904	87.4919	178	1.58428	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
20	125	450.285	484.34	113	1.74057	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
21	126	454.785	100.813	107	1.53742	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
22	127	456.12	159.5	100	1.27169	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
23	128	461.271	117.424	133	1.7008	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
24	129	463.34	101.399	257	1.70716	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
25	130	472.742	291.325	101	1.12911	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
26	131	473.407	138.476	145	1.23853	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
27	132	473.513	342.366	116	1.10283	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
28	133	479.45	22.1653	109	1.42045	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
29	134	485.404	60.2073	164	1.41513	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
30	135	490.387	164.205	63	1.55756	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
31	136	490.58	254.707	174	1.40811	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
32	137	496.026	313.187	62	2.09018	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
33	138	497.029	311.5	208	1.10594	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
34	139	498.217	292.708	145	1.10594	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
35	140	501.219	258.104	115	1.10594	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
36	141	505.109	502.192	91	1.00155	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
37	142	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
38	143	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
39	144	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
40	145	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
41	146	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
42	147	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
43	148	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
44	149	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
45	150	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
46	151	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
47	152	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
48	153	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
49	154	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
50	155	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
51	156	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
52	157	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
53	158	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
54	159	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
55	160	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
56	161	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
57	162	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
58	163	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
59	164	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
60	165	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
61	166	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
62	167	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
63	168	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
64	169	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
65	170	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
66	171	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
67	172	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
68	173	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
69	174	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
70	175	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
71	176	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
72	177	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
73	178	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
74	179	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
75	180	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
76	181	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
77	182	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
78	183	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
79	184	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
80	185	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
81	186	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
82	187	512.717	89.4565	98	1.41754	0.490916	12.9441	0.522077	0.723233	6121	31.4515	37	30
83	188	512.7											



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1	11.1154	387.587	104	1.42566	0.712037	11.5073	0.90318	0.513235	2555	39.0288	29	31
2	9.40303	429.712	66	1.20569	0.559891	9.167	0.48	0.733333	3059	46.2165	48	53
3	31.5344	452.818	77	1.17452	0.524846	9.20149	0.5227711	0.77	2990	50.4312	54	60
4	14.4113	454.546	70	1.03228	0.251764	9.4402	0.484676	0.7	3970	56.7143	68	76
5	14.3817	421.215	114	1.253	0.410824	12.0470	0.912	0.730769	3992	34.1406	39	47
6	21.18	172.38	100	1.52012	0.752151	11.2310	0.484936	0.712126	3840	29.0137	30	36
7	23.1163	198.143	140	1.26552	0.555554	13.3512	0.902326	0.631077	4072	66.709	69	81
8	25.0148	432.305	189	1.25453	0.555554	13.3512	0.902326	0.631077	4072	33.1728	37	44
9	23.0167	150.416	114	1.27031	0.414684	12.0470	0.913355	0.730769	4094	57.104	60	69
10	27.688	112.514	125	1.43321	0.721089	12.6157	0.905177	0.694441	7138	31.6391	32	39
11	27.2023	319.684	133	1.23156	0.514621	13.9311	0.912241	0.721089	4208	36.0619	37	44
12	28.6591	66.4107	113	1.23116	0.600015	11.9318	0.912241	0.721089	4208	28.352	29	35
13	30.7857	380.439	196	1.40137	0.821003	13.7913	0.903236	0.604811	5557	45.2152	45	54
14	36.7762	311.455	143	1.07865	0.315917	13.3512	0.521591	0.745714	6053	38.2131	39	46
15	49.0119	37.619	232	1.33986	0.591066	17.3123	0.921623	0.732737	1427	30.8551	32	39
16	45.7623	123.623	132	1.10508	0.435006	13.4634	0.917293	0.782051	6452	41.3812	42	50
17	46.7836	202.731	134	1.40138	0.741987	13.6619	0.917749	0.69261	8101	37.5761	37	44
18	50.7117	66.5516	212	1.48418	0.714626	16.8284	0.910931	0.69597	3441	31.3797	32	39
19	53.2043	451.719	92	1.47718	0.721555	10.823	0.910916	0.733333	18800	21.5167	22	27
20	46.0732	142.192	478	1.03578	0.577992	12.3608	0.495572	0.710059	4070	35.7059	36	43
21	46.5132	331.408	120	1.21101	0.577992	12.3608	0.495572	0.710059	4070	27.5312	28	34
22	49.3001	270.181	221	1.54955	0.780155	16.7744	0.934411	0.734667	7931	30.951	32	38
23	46.7156	339.358	136	1.54808	0.783371	16.0935	0.922077	0.737679	4288	36.1518	37	44
24	46.3913	50.0744	144	2.23108	0.439226	13.5406	0.907568	0.8	2904	28.7873	29	35
25	47.2043	431	93	1.23132	0.610764	10.8813	0.905913	0.650035	2738	40.2843	41	48
26	72.4038	287.815	117	1.41204	0.730491	12.2053	0.905917	0.643837	3541	21.4517	22	28
27	72.302	401.914	199	2.01167	0.871693	15.3126	0.917616	0.643837	5541	29.3175	30	36
28	76.5716	134.731	134	1.70184	0.801651	13.0419	0.911545	0.697917	4157	36.5234	37	44
29	74.6636	108.148	107	1.23657	0.590119	11.672	0.90678	0.704252	4122	28.7873	29	35
30	65.2589	188.102	147	2.32163	0.905811	13.4609	0.90184	0.668182	4231	36.883	38	44
31	64.292	261.741	113	1.15451	0.513778	11.9510	0.904	0.721359	4168	36.9032	37	43
32	95.8049	201.4	403	1.49138	0.721018	21.4511	0.846439	0.645913	16872	37.1435	38	44
33	90.5594	140.101	109	1.51131	0.721416	11.7866	0.892143	0.61881	4053	51.1372	52	60
34	90.5458	443.519	278	1.14168	0.432009	10.4619	0.877916	0.586644	13953	32.0057	33	39
35	95.1127	247.923	259	1.4148	0.788176	10.5115	0.937204	0.644211	4570	27.9081	28	34
36	98.2714	37.556	277	1.4148	0.82355	10.78	0.892146	0.502712	7710	27.9081	28	34
37	98.7634	144.432	93	2.13058	0.801352	10.8817	0.895714	0.650035	3656	41.4624	42	50
38	100.512	493.489	203	2.33482	0.875726	16.1158	0.913111	0.721806	628	22.5356	23	29
39	112.74	401.38	150	1.6125	0.784617	12.1348	0.913111	0.721806	628	51.9533	53	61
40	111.812	222.761	117	1.55283	0.784617	12.1348	0.865971	0.743708	4458	38.1026	39	45
41	113.865	346.671	149	1.38004	0.698829	13.7734	0.865971	0.743708	4458	28.6094	30	36
42	120.43	411.338	106	1.31794	0.65137	11.4744	0.892143	0.61881	3923	37.0094	37	43
43	129.928	192.142	84	1.15603	0.50172	10.3618	0.892143	0.61881	3923	32.0057	33	39
44	137.19	434.365	232	1.89597	0.845956	17.9155	0.925226	0.643718	5137	27.35	28	34
45	141.141	215.44	318	2.04123	0.871693	13.7013	0.916675	0.690004	4210	27.1475	28	34
46	141.141	215.44	318	1.50812	0.718552	10.1355	0.927719	0.651618	4147	35.1711	36	42
47	132.058	247.728	107	1.32113	0.661311	12.2032	0.913395	0.77727	3384	20.1174	21	26
48	145.347	17.0986	207	1.71589	0.82535	14.2345	0.924107	0.701082	6312	41.1589	41	48
49	151.728	413.183	115	1.63246	0.796105	12.1005	0.927719	0.69073	4910	35.1204	37	43
50	159.623	276.456	136	1.66378	0.841056	13.1559	0.937931	0.747253	4230	31.1023	31	37
51	159.706	91.2327	243	2.0094	0.87377	17.4619	0.924528	0.732846	9313	36.3796	37	43
52	159.35	325.024	93	1.4046	0.70128	10.28	0.912088	0.691667	3838	46.241	47	55
53	166.943	500.376	175	1.63183	0.864617	14.2271	0.916233	0.643837	4480	23.6	24	30
54	170.633	251.131	175	1.34638	0.645975	21.4511	0.910156	0.701885	14347	38.792	39	45
55	171.162	34.2373	167	1.52018	0.731181	14.5819	0.912349	0.701611	5095	30.309	32	38
56	176.5	387.358	95	1.20378	0.540326	10.9391	0.845218	0.61519	9007	40.2084	40	46
57	182.352	635.777	224	2.0037	0.828322	16.481	0.915406	0.745192	4011	29.7179	30	36
58	179.327	659.119	101	1.22441	0.578318	11.3101	0.845218	0.61519	9007	21.4512	22	28
59	183.359	190.781	120	1.23717	0.581013	12.7482	0.845218	0.61519	9007	37.5761	37	43
60	185.444	503.145	110	1.46453	0.841013	13.4345	0.845218	0.61519	9007	37.5761	37	43
61	197.016	139.323	223	1.43283	0.790337	14.4503	0.845218	0.61519	9007	29.3477	30	36
62	191.681	214.072	130	2.31337	0.891237	13.2553	0.907893	0.746667	4016	35.7148	36	42
63	199.681	655.04	115	1.31204	0.611682	11.9510	0.91129	0.641667	4016	34.2097	35	41
64	207.119	289.204	226	1.53022	0.833202	14.9633	0.906835	0.742319	7354	33.9744	34	40
65	203.444	35.0171	117	1.46158	0.693669	12.2033	0.92126	0.75971	3375	37.5761	37	43
66	206.22	259.208	174	1.34482	0.680437	15.0345	0.92278	0.705319	6739	37.5761	37	43
67	210.104	15.8125	120	1.45902	0.721174	12.7462	0.90108	0.711111	4017	31.3828	32	38
68	220.184	214.747	161	1.13172	0.471599	14.2273	0.92126	0.742444	18142	26.4316	27	33
69	225.176	214.747	161	1.11992	0.44119	14.4042	0.92126	0.742444	18142	26.4316	27	33
70	230.979	215.594	144	1.33137	0.45216	13.4343	0.902726	0.695238	4134	28.2131	29	35
71	224.473	653.272	146	2.32131	0.903331	20.9391	0.900276	0.618711	10802	31.2187	32	38
72	235.502	137.459	235	1.7444	0.820288	16.0181	0.929417	0.643939	9318	34.9715	36	42
73	221.124	393.719	89	1.45186	0.721978	10.6151	0.89959	0.710644	4314	48.4719	47	55
74	242.141	155.406	251	2.40019	0.884397	17.4769	0.92061	0.693265	9132	36.3828	38	44
75	239.34	189.286	162	1.71597	0.820584	14.2519	0.90011	0.692308	4164	25.4272	26	32
76	238.514	21.8451	138	1.77073	0.825269	13.2555	0.93	0.631113	4010	26.5152	27	33

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1	1	21.2168	364.125	54	1.29161	0.431372	9.92703	0.471712	0.472722	2612	56.9063	57	44	66
2	2	24.0233	453.946	129	1.42866	0.421221	12.8158	0.721439	0.615138	3172	30.0135	30	23	37
3	3	35.6828	116.393	140	1.72155	0.423512	13.2512	0.309031	0.729167	4213	30.8071	31	23	38
4	4	39.9772	251.374	206	1.6479	0.79183	14.1953	0.319412	0.471432	5014	42.7533	42	35	53
5	5	36.5362	228.443	278	1.66005	0.331795	15.0915	0.327003	0.391111	9196	31.6079	45	28	64
6	6	39.3667	41.57	150	1.17573	0.327089	13.8198	0.320215	0.653306	4709	31.3923	33	28	58
7	7	36.3765	362.188	85	1.17688	0.427265	10.4021	0.494737	0.709233	3980	66.8235	49	29	57
8	8	45.323	34.1727	139	1.40418	0.703172	13.3031	0.358621	0.648194	4279	30.7612	32	25	34
9	9	51.3216	101.948	107	1.71145	0.625124	15.4201	0.312195	0.678457	4110	33.5019	24	18	29
10	10	55.8094	467.951	103	1.41519	0.745675	11.4143	0.314031	0.693238	4298	28.4341	30	22	36
11	11	55.5416	176.373	124	1.31497	0.450118	11.4146	0.3	0.693209	4038	32.5130	33	23	40
12	12	65.4189	170.964	314	2.47752	0.314123	20.3108	0.73903	0.497142	16200	50	52	39	61
13	13	61.6415	297.138	195	1.65939	0.794019	15.3157	0.319811	0.314214	9035	44.2021	48	31	57
14	14	61.3164	214.022	160	1.77726	0.426578	15.1348	0.301523	0.314286	8770	40.5	50	37	59
15	15	71.2272	315.174	43	1.42958	0.711415	7.5614	0.313263	0.314286	3368	71.9	74	56	94
16	16	71.3337	227.052	153	1.43509	0.710039	13.5572	0.321687	0.72	4977	29.2748	30	24	35
17	17	76.3015	352.453	53	1.41916	0.491634	12.1472	0.312793	0.726111	3214	61.2019	68	50	80
18	18	76.3015	352.453	53	1.41916	0.491634	12.1472	0.312793	0.726111	3214	61.2019	68	50	80
19	19	81.9479	94.3466	182	1.21769	0.421756	11.6352	0.323017	0.7161905	8603	61.4394	67	27	54
20	20	81.9479	94.3466	182	1.21769	0.421756	11.6352	0.323017	0.7161905	8603	61.4394	67	27	54
21	21	86.0609	317.826	115	1.25503	0.404248	11.1003	0.312499	0.746753	1041	35.2	36	27	43
22	22	86.0609	317.826	115	1.25503	0.404248	11.1003	0.312499	0.746753	1041	35.2	36	27	43
23	23	95.6796	190.528	197	1.41488	0.444904	11.6376	0.320561	0.724255	3240	61.9026	41	34	59
24	24	95.6796	190.528	197	1.41488	0.444904	11.6376	0.320561	0.724255	3240	61.9026	41	34	59
25	25	95.6796	190.528	197	1.41488	0.444904	11.6376	0.320561	0.724255	3240	61.9026	41	34	59
26	26	95.6796	190.528	197	1.41488	0.444904	11.6376	0.320561	0.724255	3240	61.9026	41	34	59
27	27	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
28	28	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
29	29	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
30	30	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
31	31	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
32	32	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
33	33	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
34	34	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
35	35	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
36	36	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
37	37	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
38	38	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
39	39	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
40	40	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
41	41	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
42	42	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
43	43	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
44	44	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
45	45	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
46	46	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
47	47	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
48	48	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
49	49	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
50	50	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
51	51	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
52	52	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
53	53	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
54	54	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
55	55	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
56	56	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
57	57	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
58	58	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
59	59	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
60	60	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
61	61	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
62	62	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
63	63	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
64	64	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
65	65	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
66	66	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
67	67	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
68	68	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
69	69	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
70	70	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
71	71	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
72	72	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
73	73	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
74	74	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
75	75	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
76	76	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
77	77	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
78	78	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
79	79	102.493	121.97	203	2.4942	0.321632	16.0769	0.433391	0.461364	9738	31.0413	42	31	53
80	80	102.493	121.97	203	2.4942	0.321632	1							

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EV Table 2.doc

Example of the summary output of AnalyseDNA.m program  
(summary for 10 3 by 3 montage images)

1	1107	163.812	78.3518	1.58819	0.388735	0.724461	0.137986	11.0612	1.319	0.305227	0.0250361	0.707218	0.031176	6448.26	3186.35	61.539	18.352	62.3616	18.9393
2	21.4103	16.336	50.5806	22.4584	0.388942	0.727835	0.138162	11.2634	3.43288	0.306571	0.0211453	0.70177	0.0720289	6786.37	3885.1	62.3416	17.0645	63.8245	17.563
3	1795	169.034	85.8722	1.48511	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
4	21.0245	16.336	51.3109	21.2507	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
5	1795	169.034	85.8722	1.48511	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
6	21.0245	16.336	51.3109	21.2507	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
7	1795	169.034	85.8722	1.48511	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
8	21.0245	16.336	51.3109	21.2507	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
9	1795	169.034	85.8722	1.48511	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287
10	21.0245	16.336	51.3109	21.2507	0.387935	0.725532	0.137643	11.0853	3.33804	0.303247	0.0241822	0.702891	0.0720005	6891.06	3575.81	64.2167	20.5816	66.3816	21.2287

CLAIMS

What is claimed is:

1. A method of predicting a property of a manipulation of cells based  
5 upon a descriptor, said method comprising:  
    providing a plurality of cells;  
    manipulating said plurality of cells;  
    capturing a morphological value from said plurality of cells;  
    assigning a degree of presence of said morphological value; and  
10     storing said morphological value and said degree of presence;  
    wherein said descriptor is derived from a first component of a cell and  
a second component of said cell, said capturing said morphometric value from said  
plurality of cells comprises determining a relationship between said first component  
and said second component.
- 15     2. The method of claim 1 wherein said first component and said second  
component are selected from a protein, a protein modification, a nucleic acid, a lipid,  
a carbohydrate, a subcellular structure and an organelle.
3. The method of 1 wherein said step of manipulation occurs in a manner  
selected from a electrical source, a chemical source, a thermal source, a gravitational  
20 source, a nuclear source, a temporal source, and a biological source
4. The method of claim 3 wherein said chemical source is selected from a  
pharmacological candidate and a drug screening library.
5. The method of claim 1 wherein said morphological value is selected  
from a count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a  
25 shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius,  
an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an  
equivalent oblate volume, an equivalent sphere surface area, an average gray value, a  
total gray value, and an optical density.
6. The method of claim 1 wherein said degree of presence is  
30 multiple of a quantized value.

7. A computer program product for populating a database with manipulated biological information, said computer program product comprising:
- code for providing a plurality of cells in various stages of the cell cycle, said stages of the cell cycle including at least one selected from interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase;
  - code for manipulating said cells in said various stages of cell cycle development to form a plurality of manipulated cells;
  - code for capturing an image of said plurality of manipulated cells;
  - code for determining a descriptor from said image for said manipulated cells;
  - code for populating a database with said descriptor;
  - wherein said image includes a first component of a cell and a second component of said cell; and
  - a computer readable storage medium for holding the codes.
8. The computer program product of claim 7 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a sub-cellular structure and an organelle.
9. The computer program product of claim 7 wherein said image is a digitized representation of said plurality of manipulated cells.
11. The computer program product of claim 9 wherein said digitized representation provides a density value of said plurality of manipulated cells.
11. The computer program product of claim 7 wherein said descriptors comprise numeric or logical values.
12. The computer program product of claim 11 wherein said values further comprises a nucleotide.
13. The computer program product of claim 11 wherein said values further comprises an amino acid letter.
14. A system for capturing images of cells or cell structures, the system comprising:
- a cell holder comprising a plurality of sites in a spatial orientation, each of the sites being capable of holding a plurality of cells to be imaged;



an image capturing device coupled to the cell holder, the image capture device being adapted to capture at least one image in at least one of the plurality of sites;

an illumination apparatus comprising a liquid light guide coupled to the plate for highlighting the plurality of cells in a relatively even spatial manner for image capturing purposes;

an image processing device coupled to the image capturing device, the image capturing device being adapted to convert the image into a digital representation; and

a database storage device comprising a database management element coupled to the image capturing device, the database storage device being adapted to retrieve the digital representation of the image from the image processing device and storing the digital representation.

15. The system of claim 14 further comprising a stage comprising a device for moving the cell holder in a spatial direction to traverse across the cell holder in the spatial orientation.

16. The system of claim 14 wherein the illumination apparatus comprises sub-elements, at least one of the sub-elements being positioned away from the image capturing device to prevent a possibility of vibration from the one sub-elements to be transmitted to the image capturing device.

17. The system of claim 14 wherein the digital representation comprises a plurality of regions and objects.

18. The system of claim 14 further comprising a computing device connected between the database storage device and the image processing device.

19. The system of claim 14 wherein the image capturing device comprises a magnification of at least 1X and greater to capture the image of the site.

20. The system of claim 14 wherein the plurality of sites comprises at least 96 sites.

21. The system of claim 14 wherein the liquid light guide characterized as a flexible member that substantially prevents vibration from the an element of the illumination apparatus to be transferred to the image capturing device.

22. The system of claim 14 wherein the spatial direction can be selected from an x-direction, a y-direction, or a z-direction in a Cartesian coordinate system.

23. The system of claim 14 wherein the each of the sites comprises a volume that is sufficient to prevent a solution therein from evaporating in a substantial manner that may influence the image capturing.

24. A method for identifying a mechanism of action for a first compound, the method comprising the steps of:

receiving the first compound;

measuring at least one feature of a cellular phenotype to define a target phenotype;

identifying additional compounds providing a feature similar to the feature identified in the measuring step; and

characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

25. The method of claim 24 comprising the further step of storing the additional compounds and their associated phenotypes in a database.

26. A method for identifying a mechanism of action for a cellular stimulus, the method comprising the steps of:

receiving cells of interest;

measuring at least one feature of the cells to define and quantify a target phenotype;

identifying additional compounds providing a feature similar to the feature identified in the measuring step; and

characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

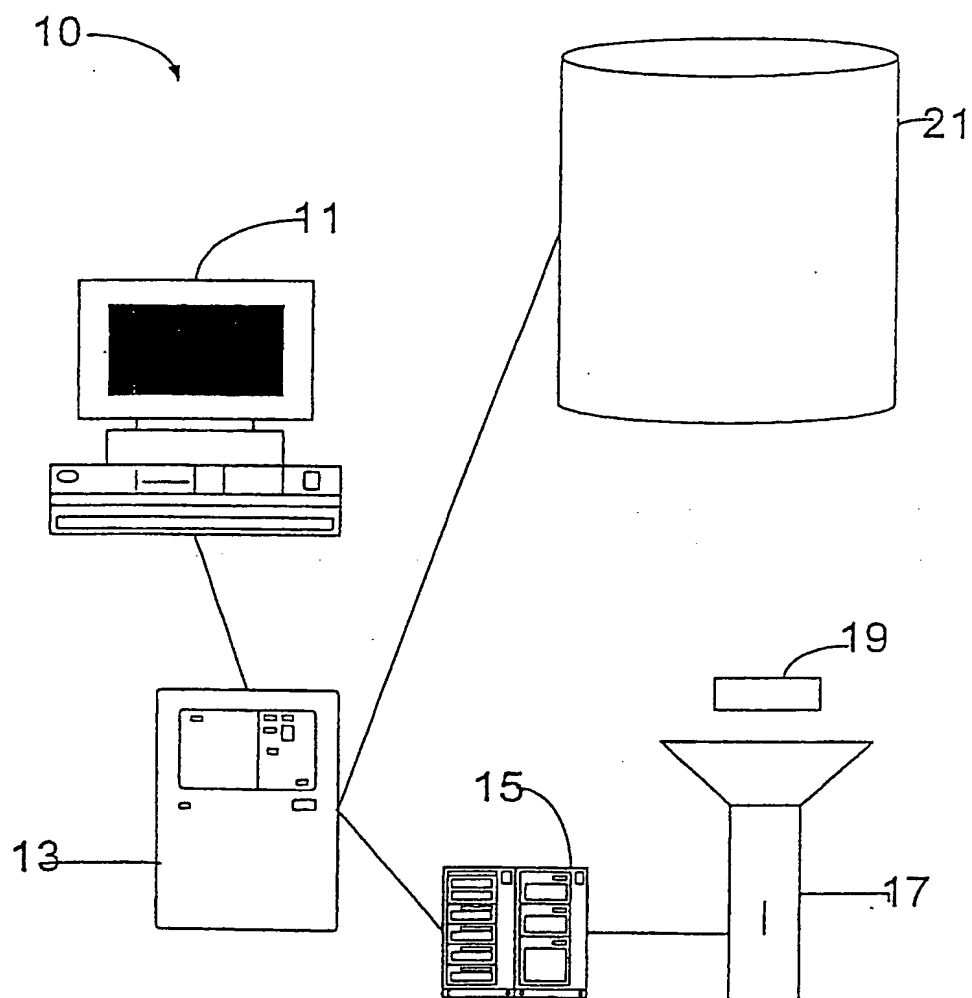
27. A method for identifying information relevant to at least one of a mechanism of action and cellular activity by utilizing assay data to elucidate a phenotype, the method comprising the steps of:

identifying a target protein;

identifying positive and negative biochemical hits related to the target protein;

defining the target phenotype utilizing the positive and negative hits;

identifying other compounds providing similar features; and  
characterizing the first compound in terms of distance from a specific  
target phenotype having known characteristics.

**FIG. 1**

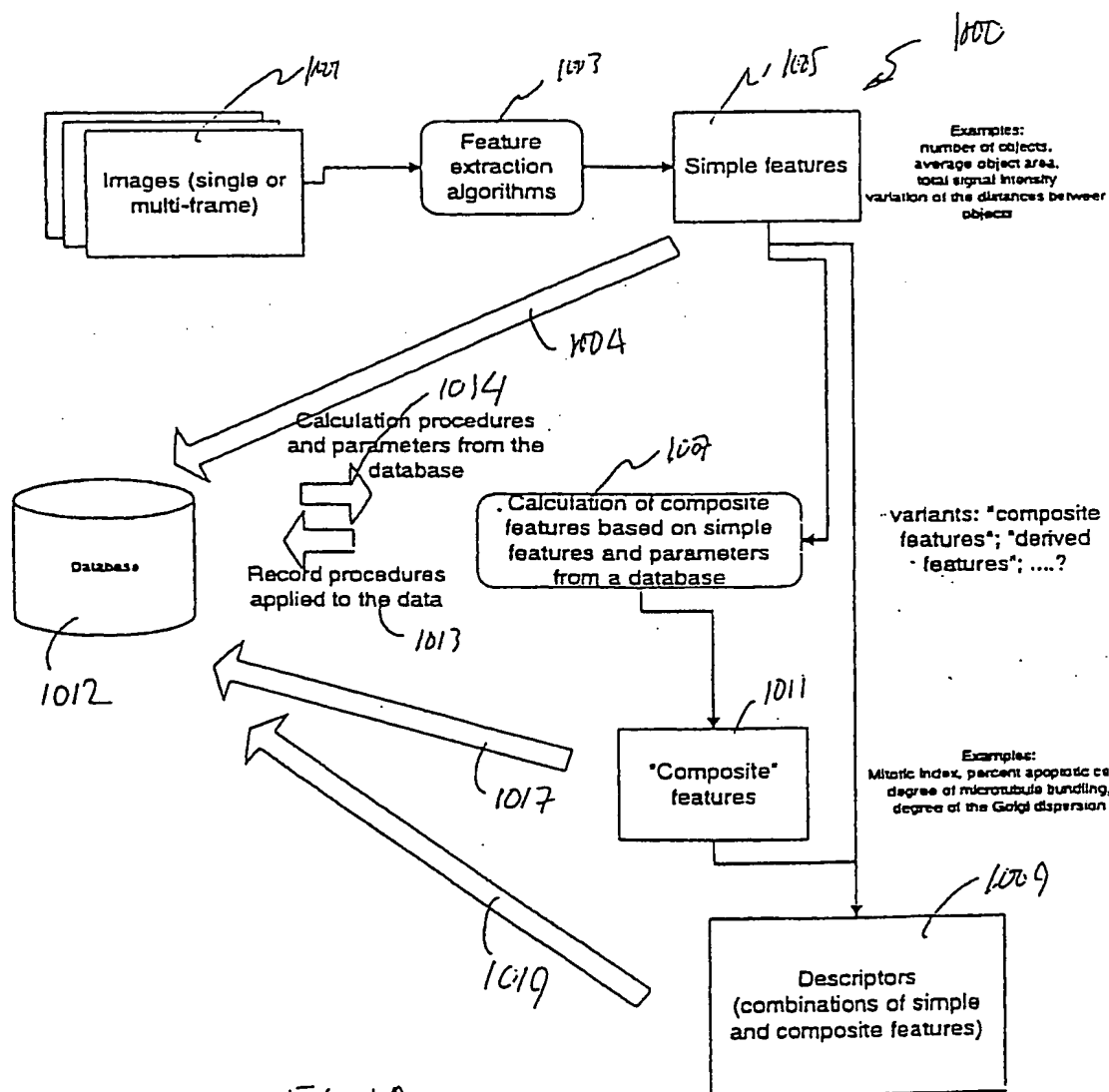


FIG. 1A

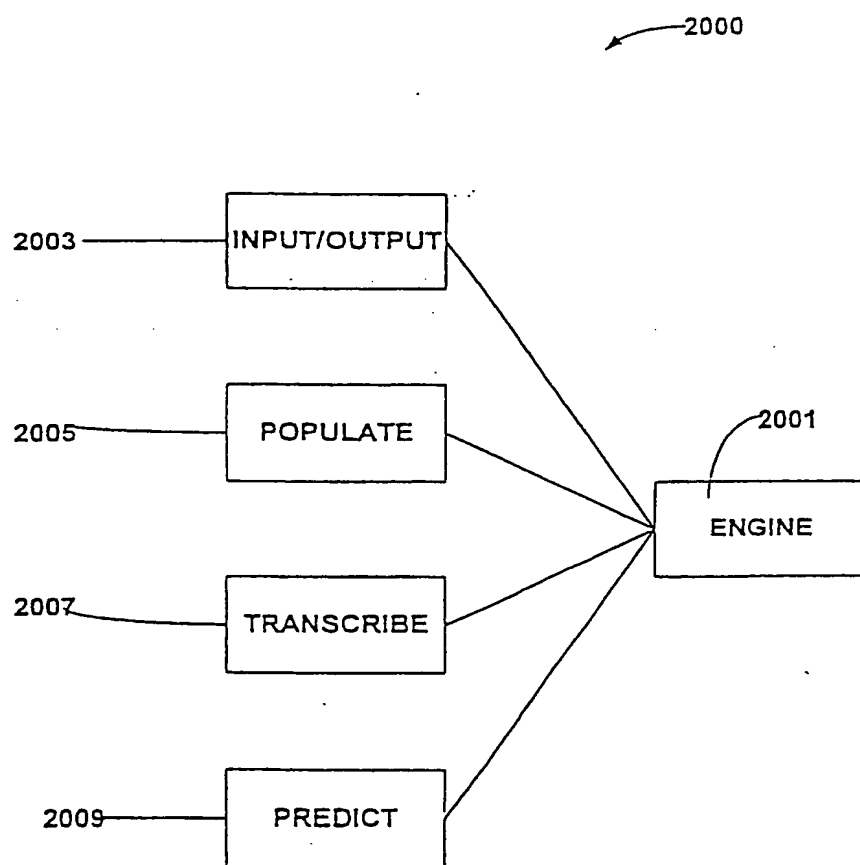


FIG. 1B

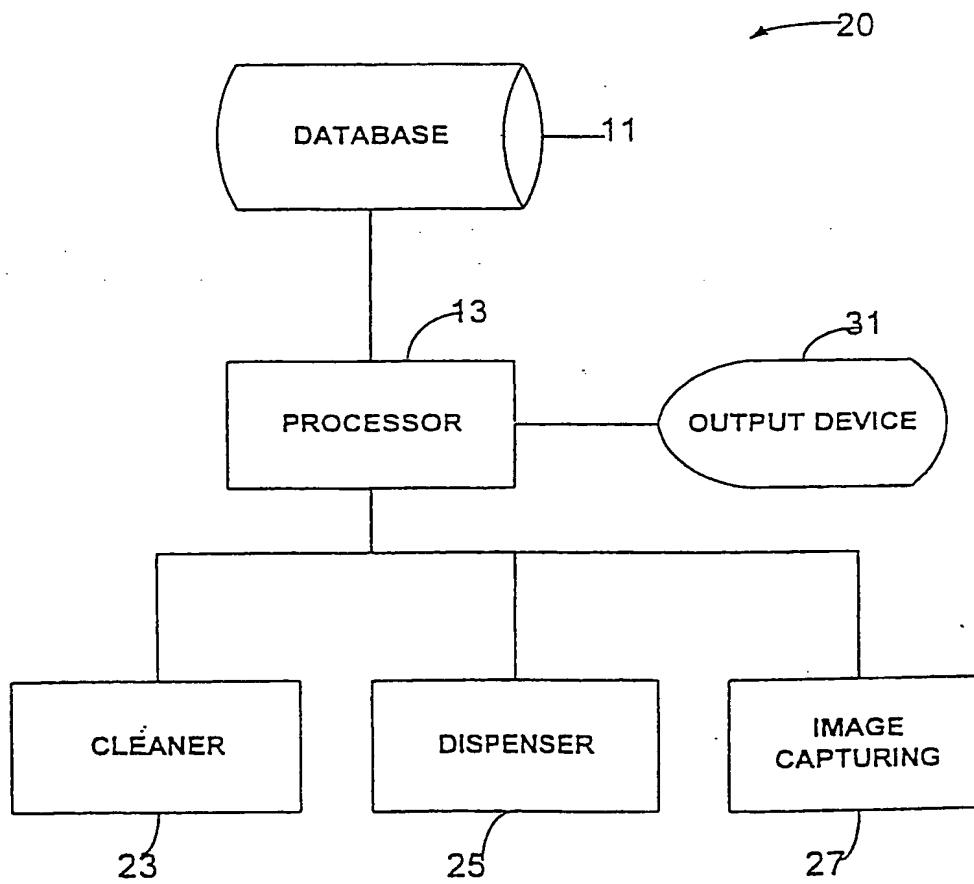
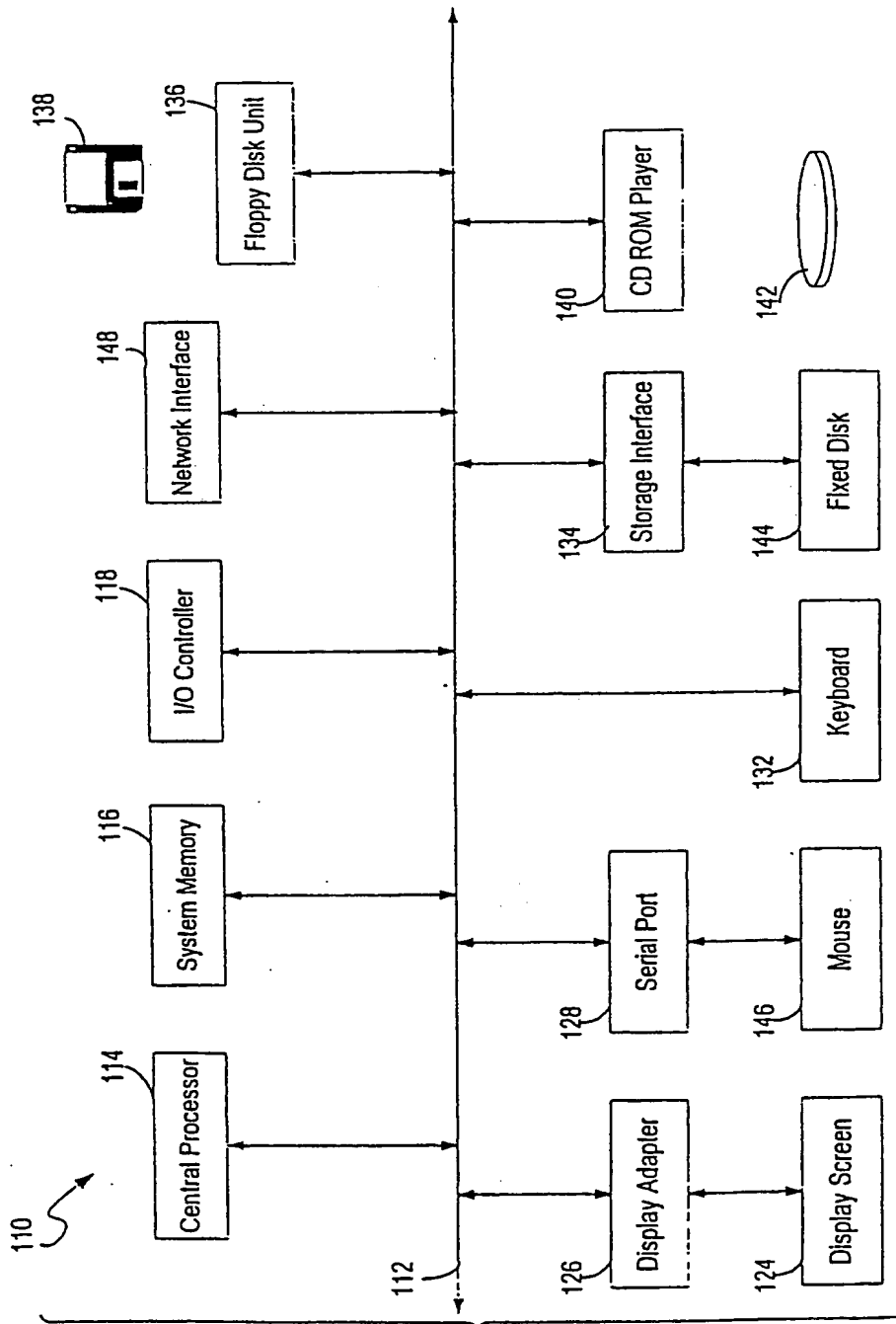


FIG. 2

*Fig 3*



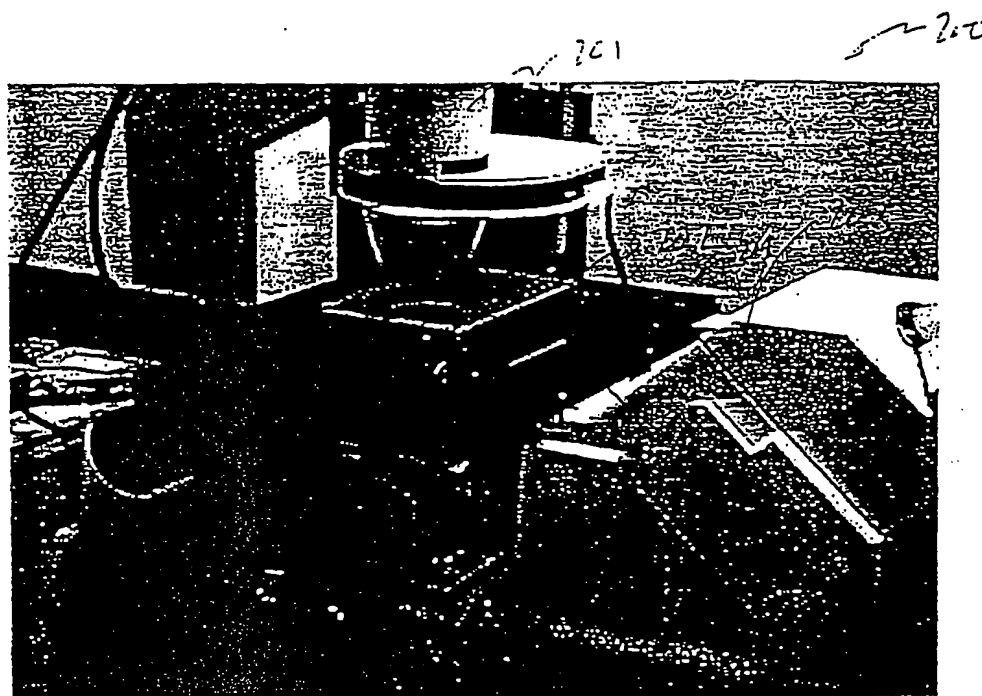


Fig. 4

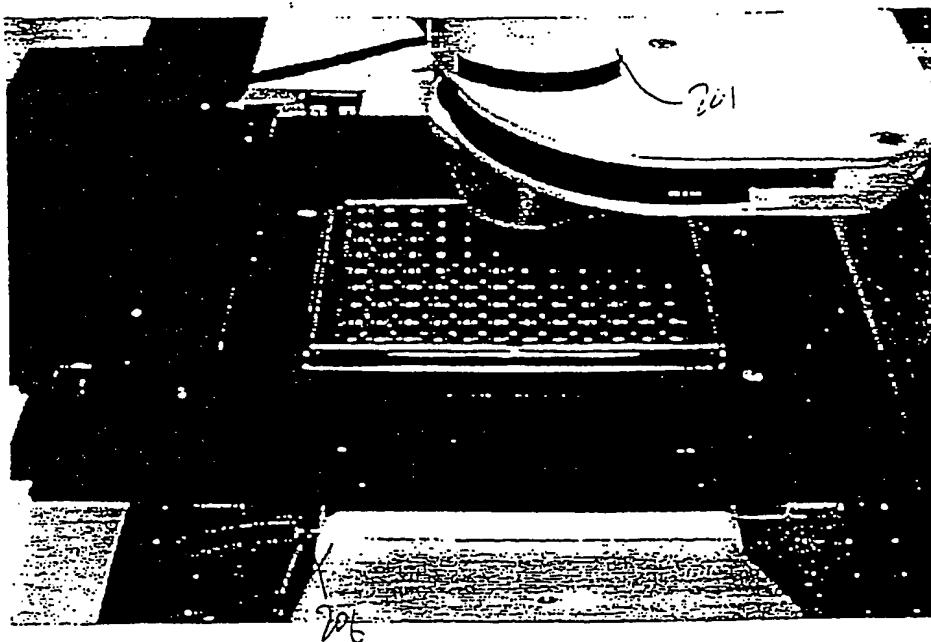


FIG 5

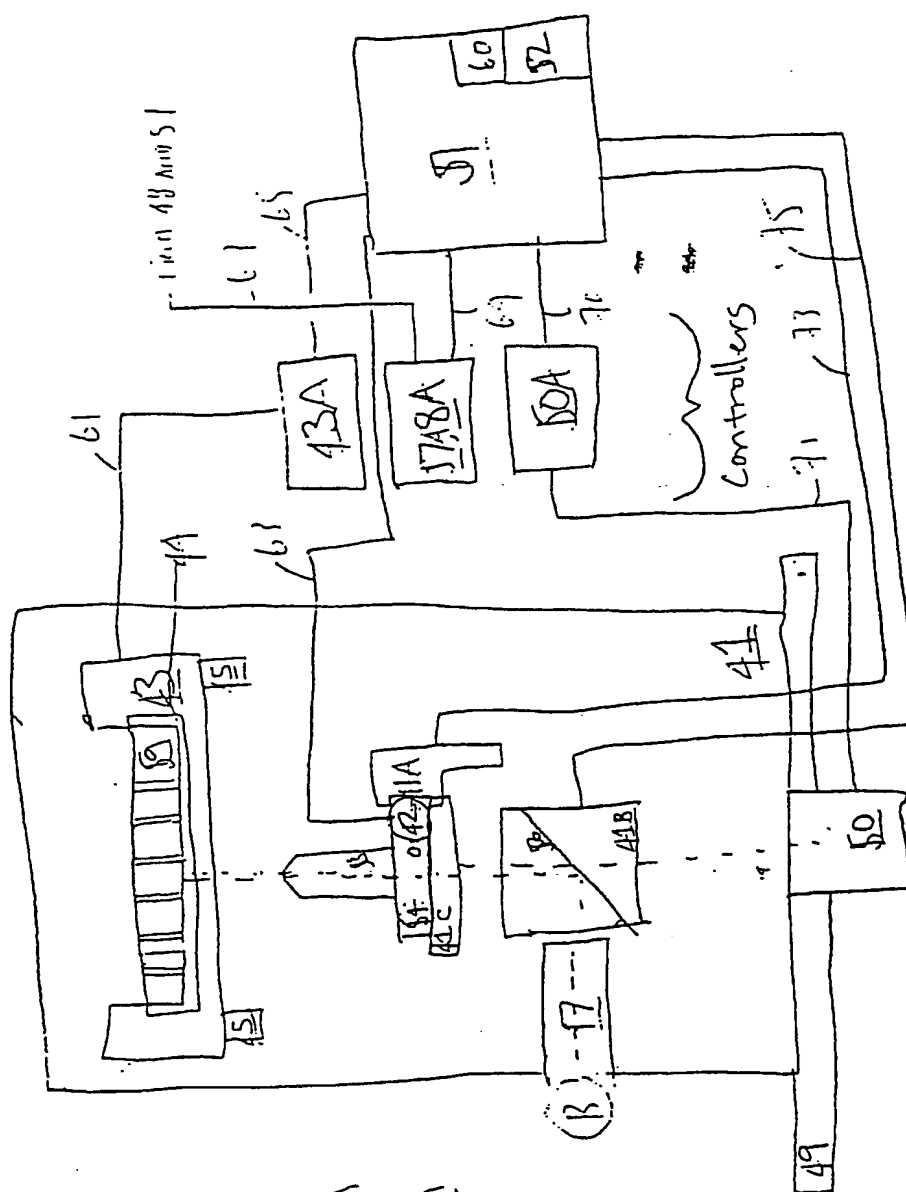


FIG. 5A

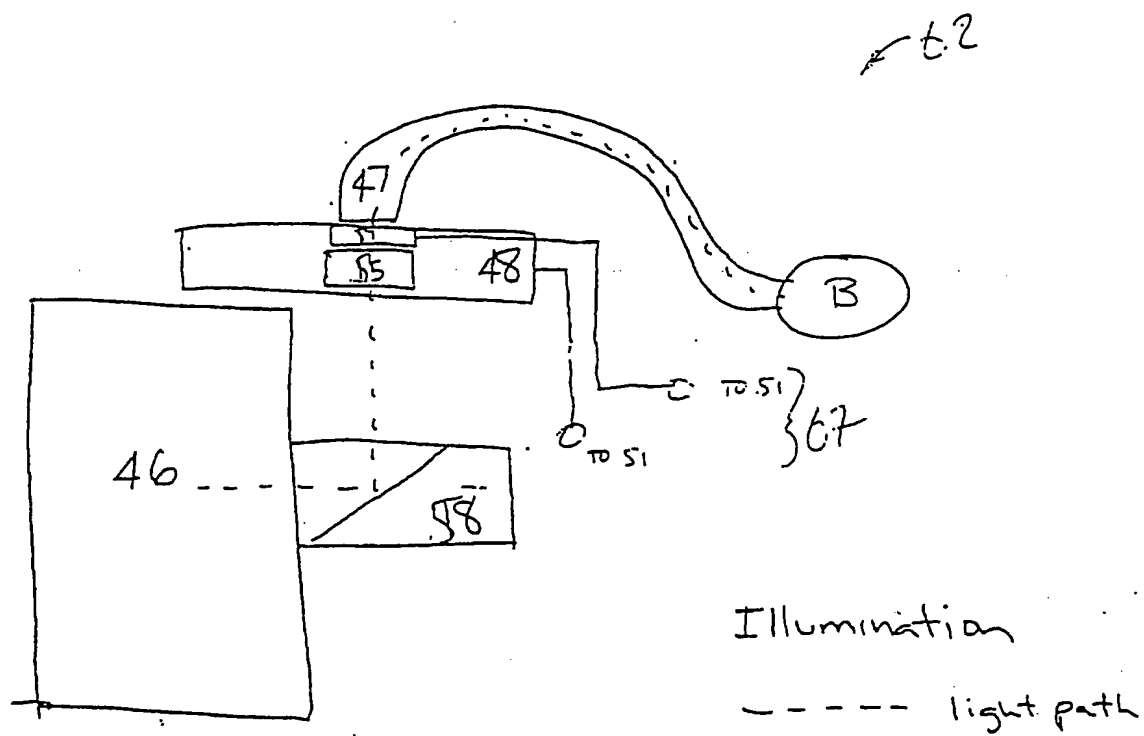


FIG. 5B

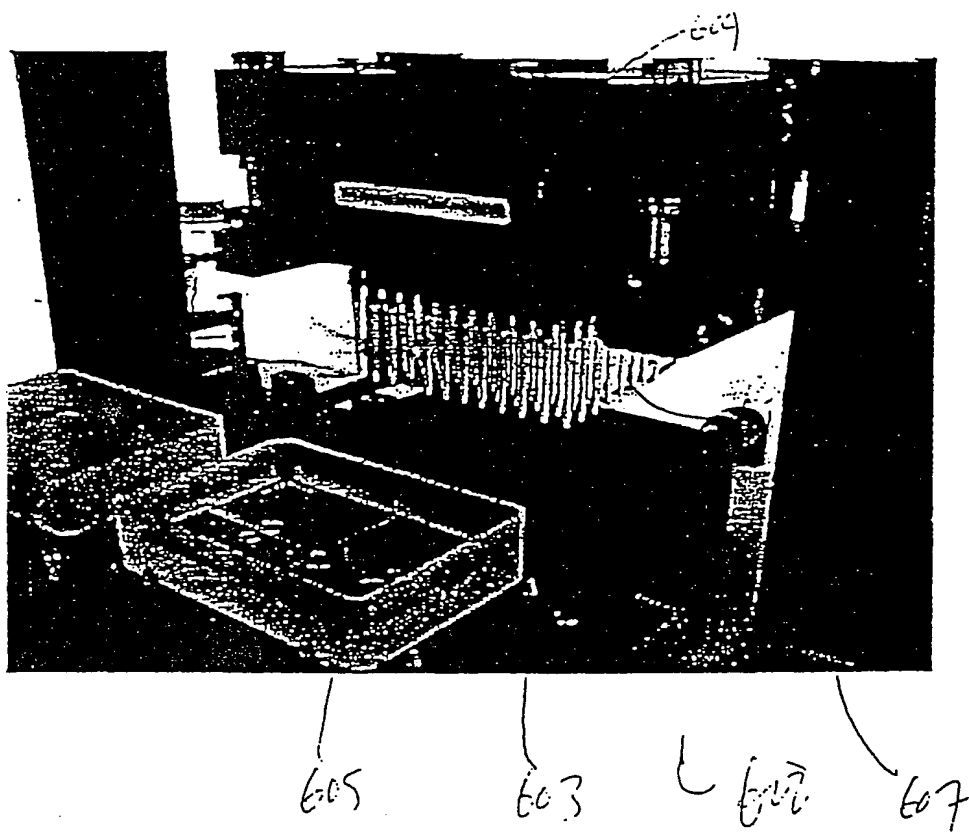


FIG. 6

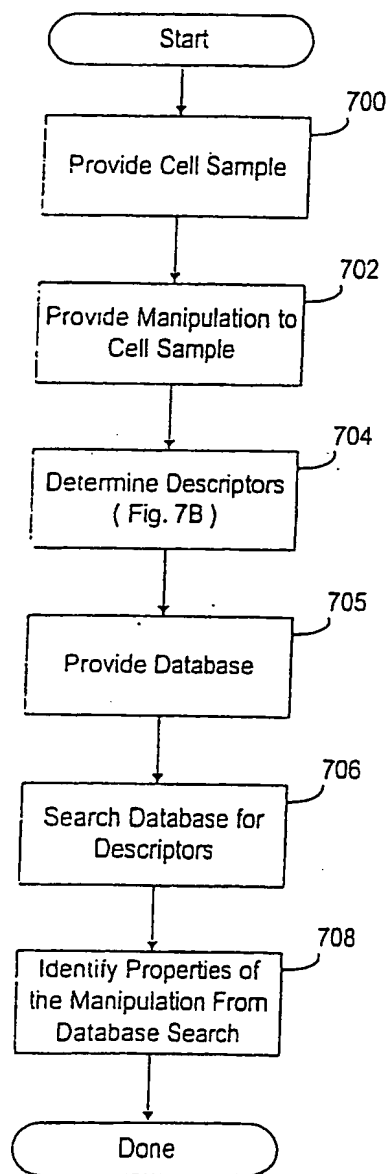


Fig. 7A

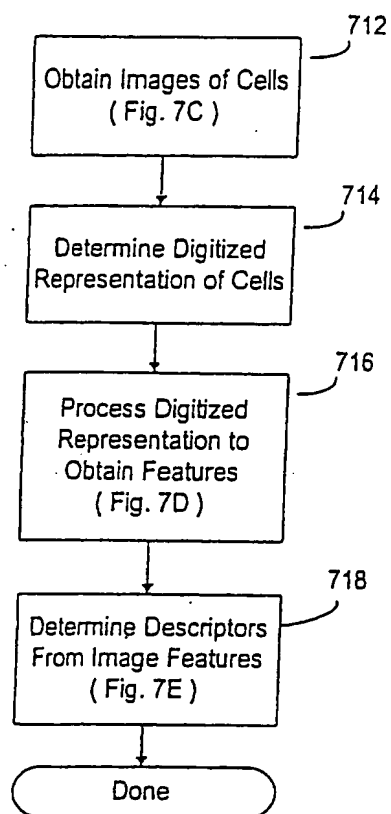


Fig. 7B  
Step 704 of Fig. 7A

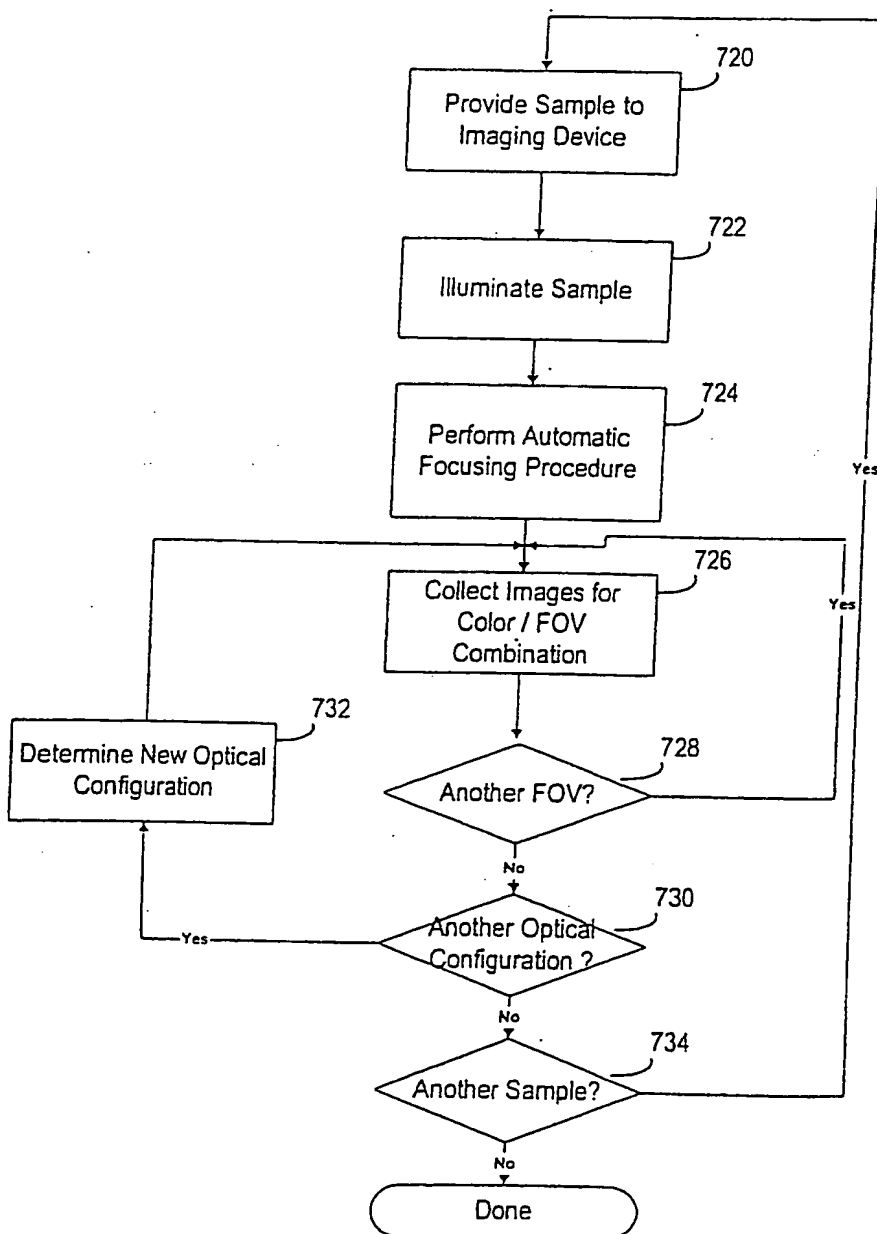


Fig. 7C  
Step 714 of Fig. 7B



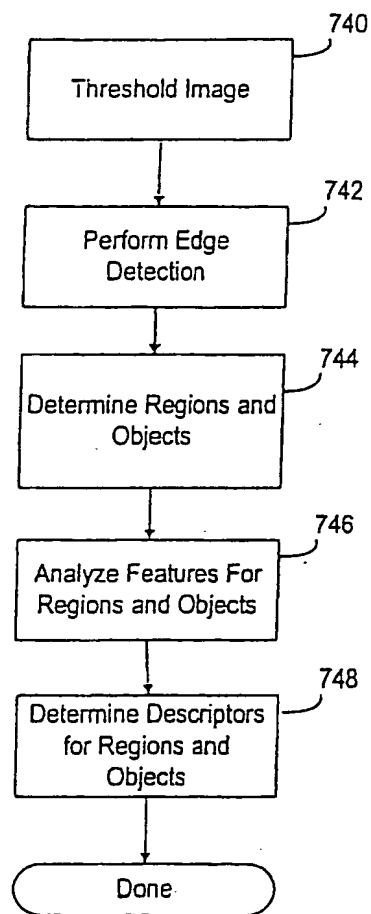


Fig. 7D  
Step 716 of Fig. 7B

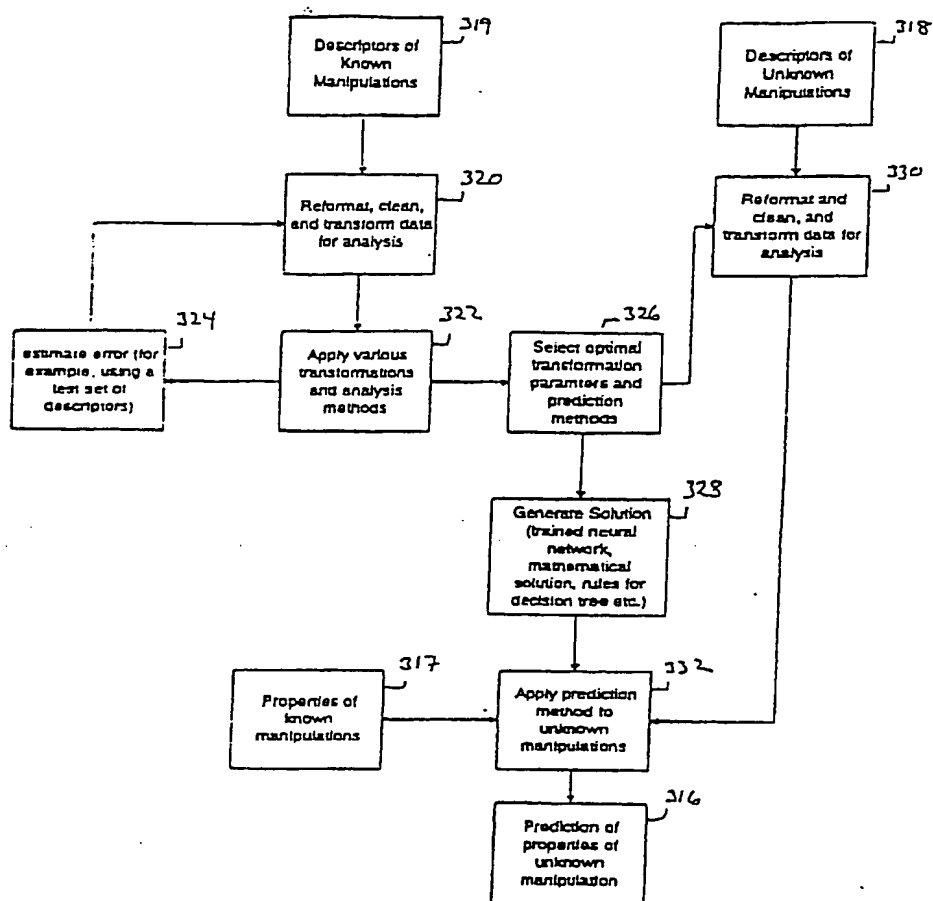


FIG. 7E

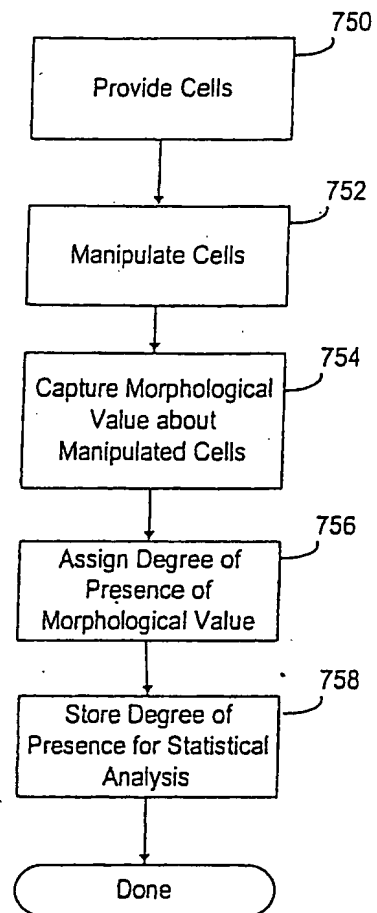


Fig. 7F

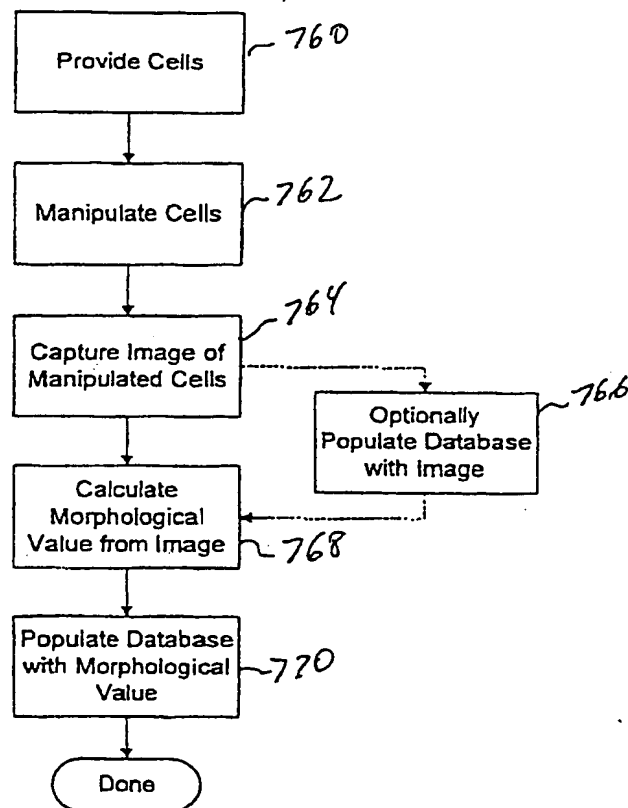


Fig 7G

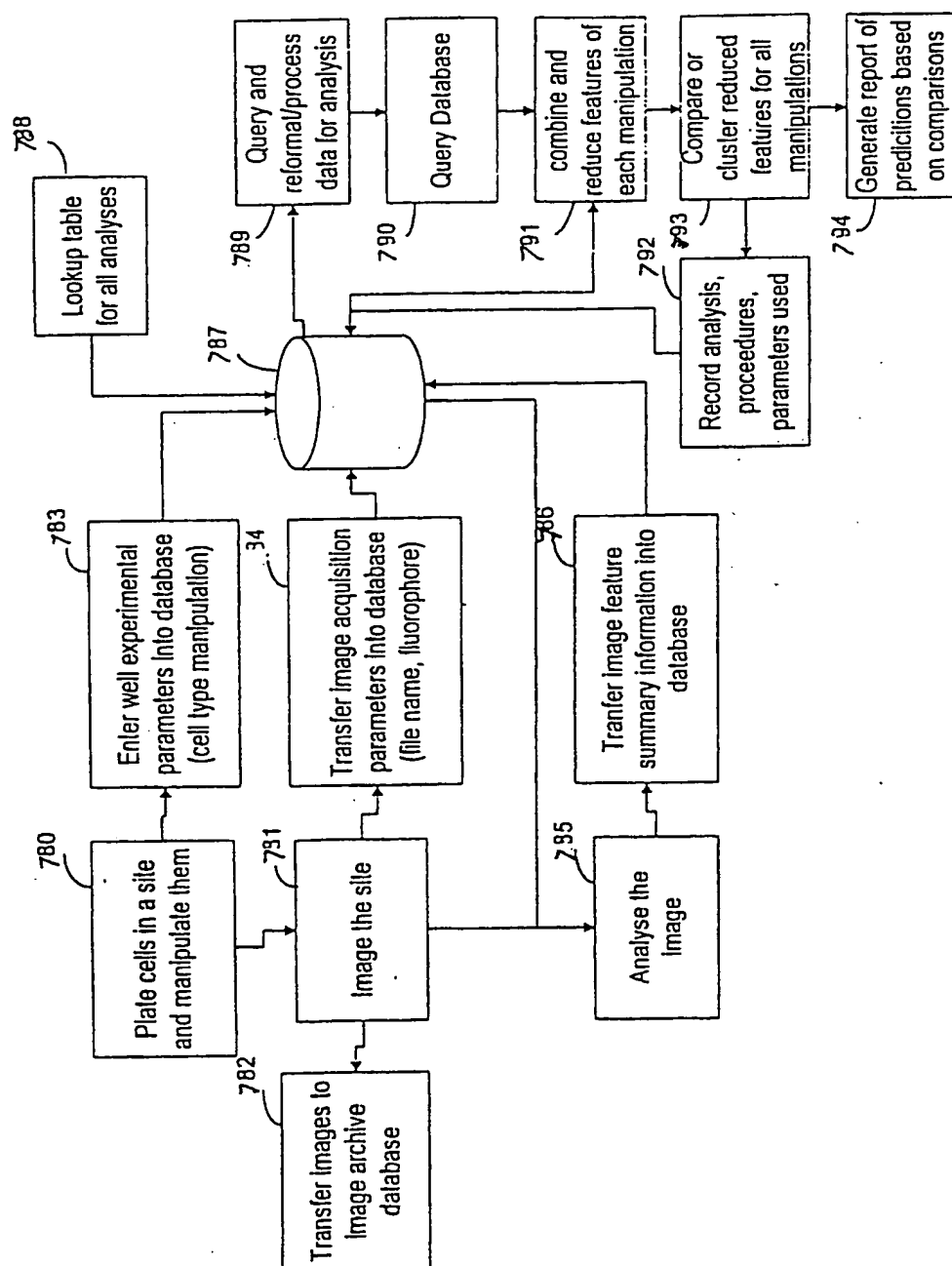


Fig. 7 H

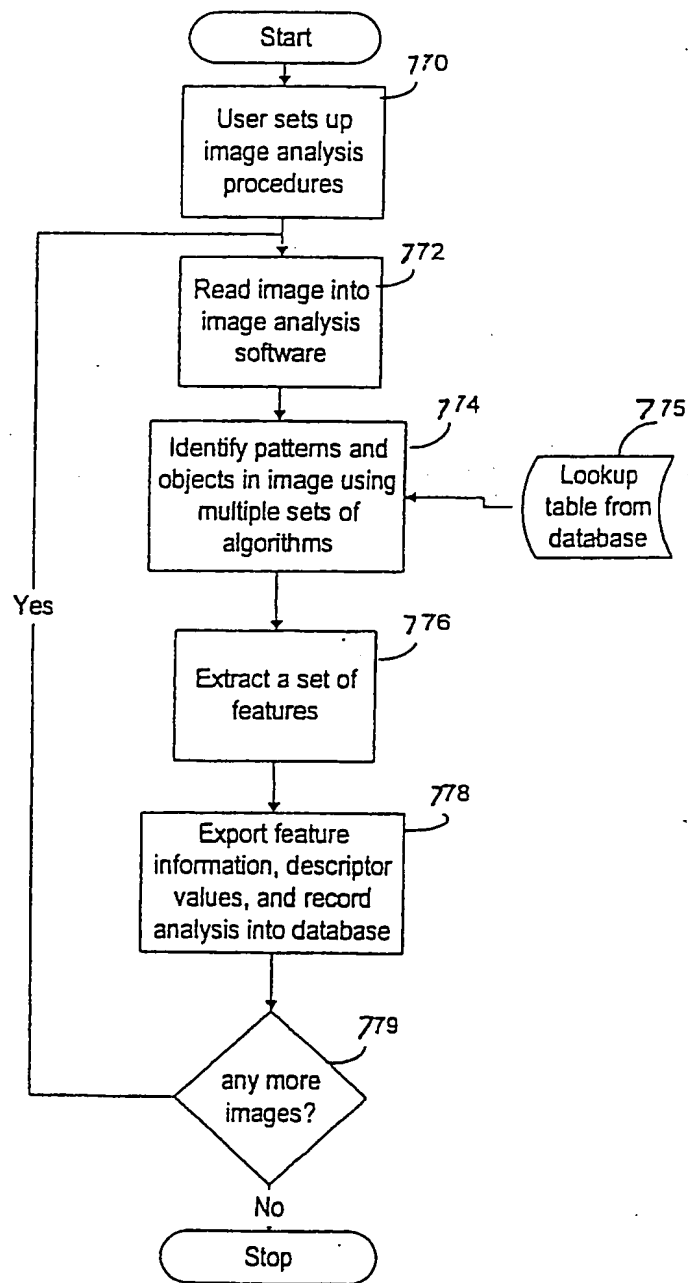


Fig. 71

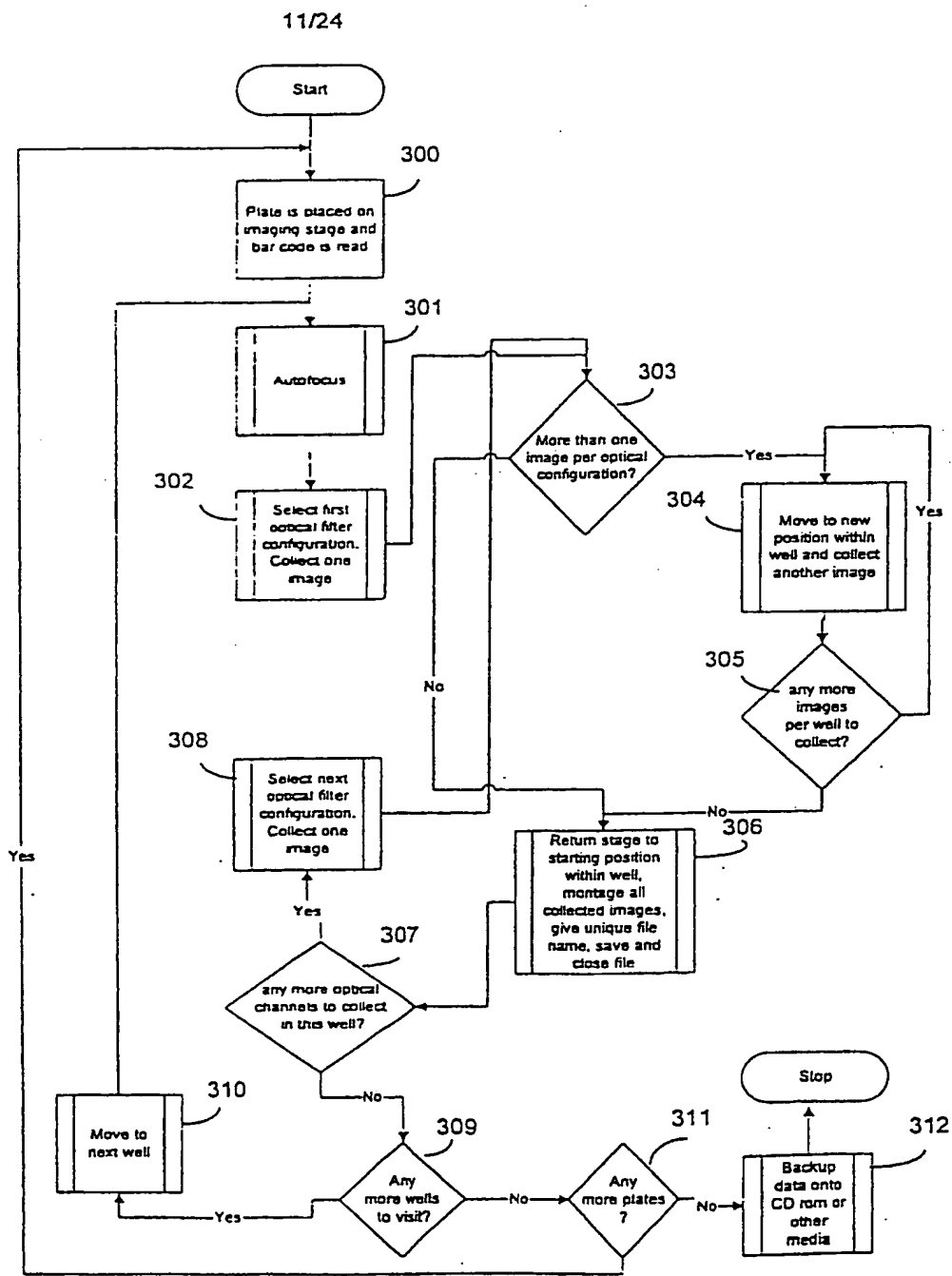


Fig. 7J

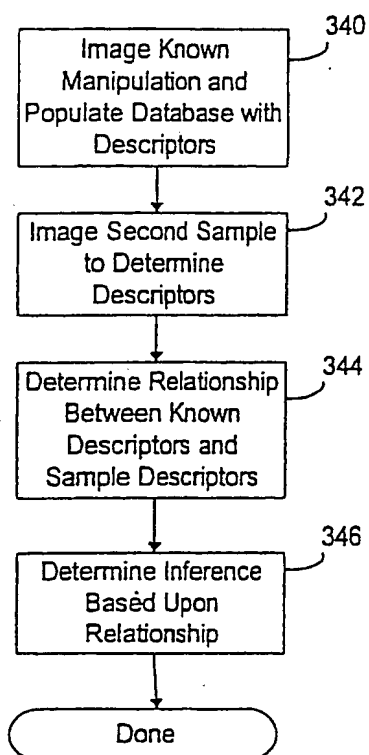


Fig. 7X



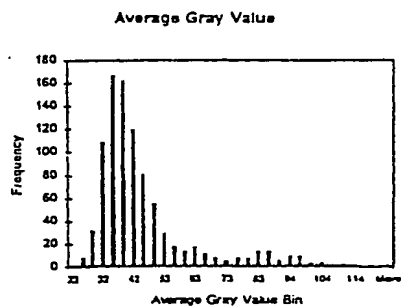


Fig. 8A

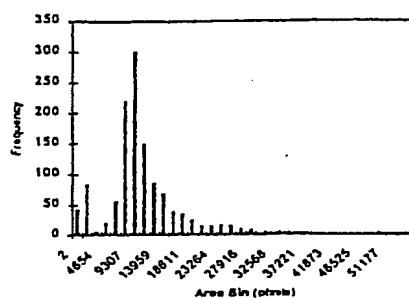


Fig. 8B

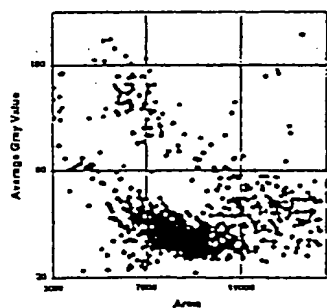


Fig. 8C

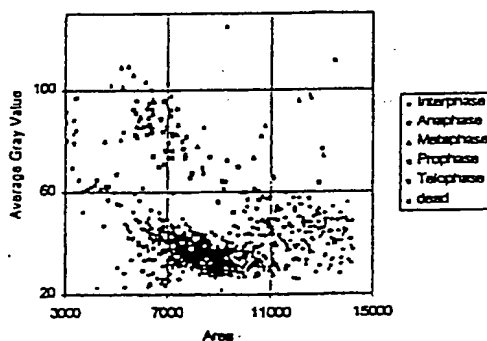


Fig. 8D

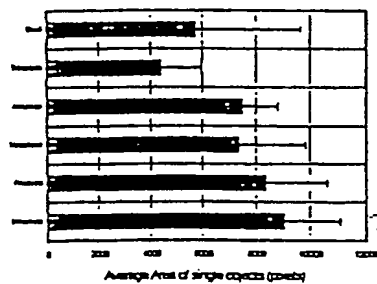


Fig. 8E

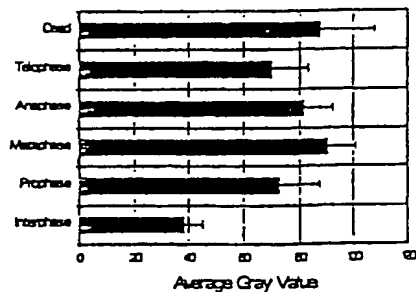


Fig. 8F

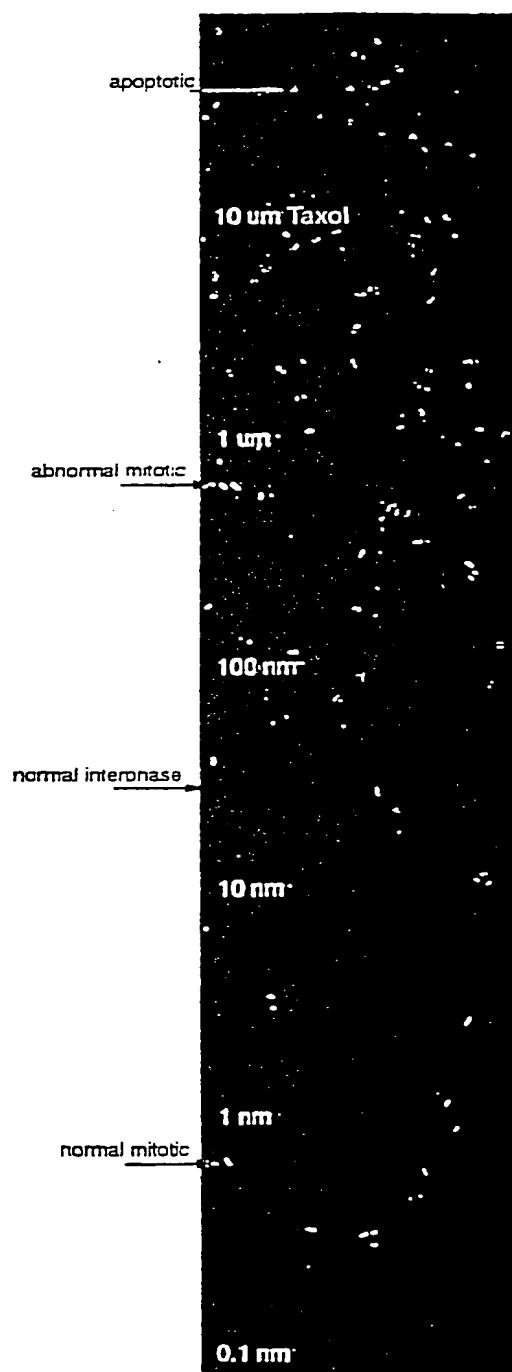


Fig. 9

MDCK cells treated with Taxol for 4.5 hours

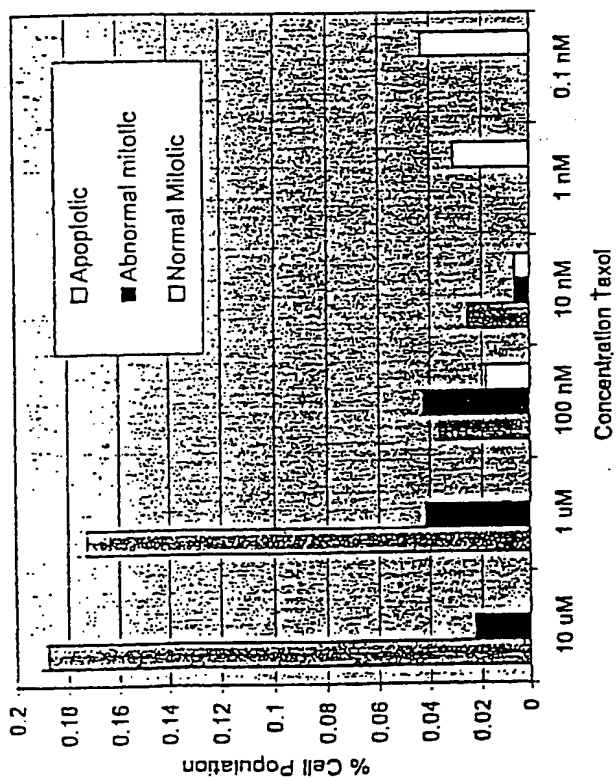
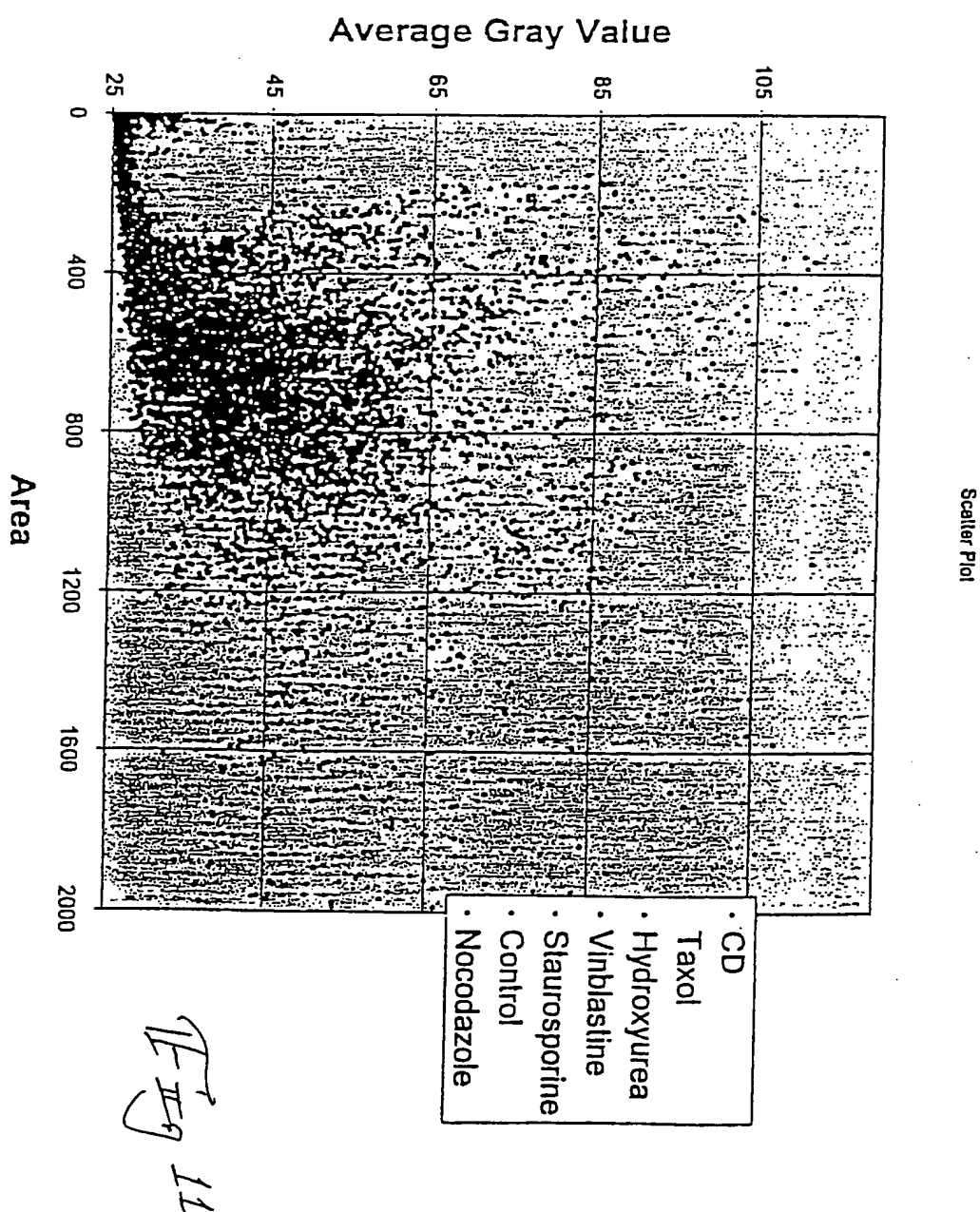
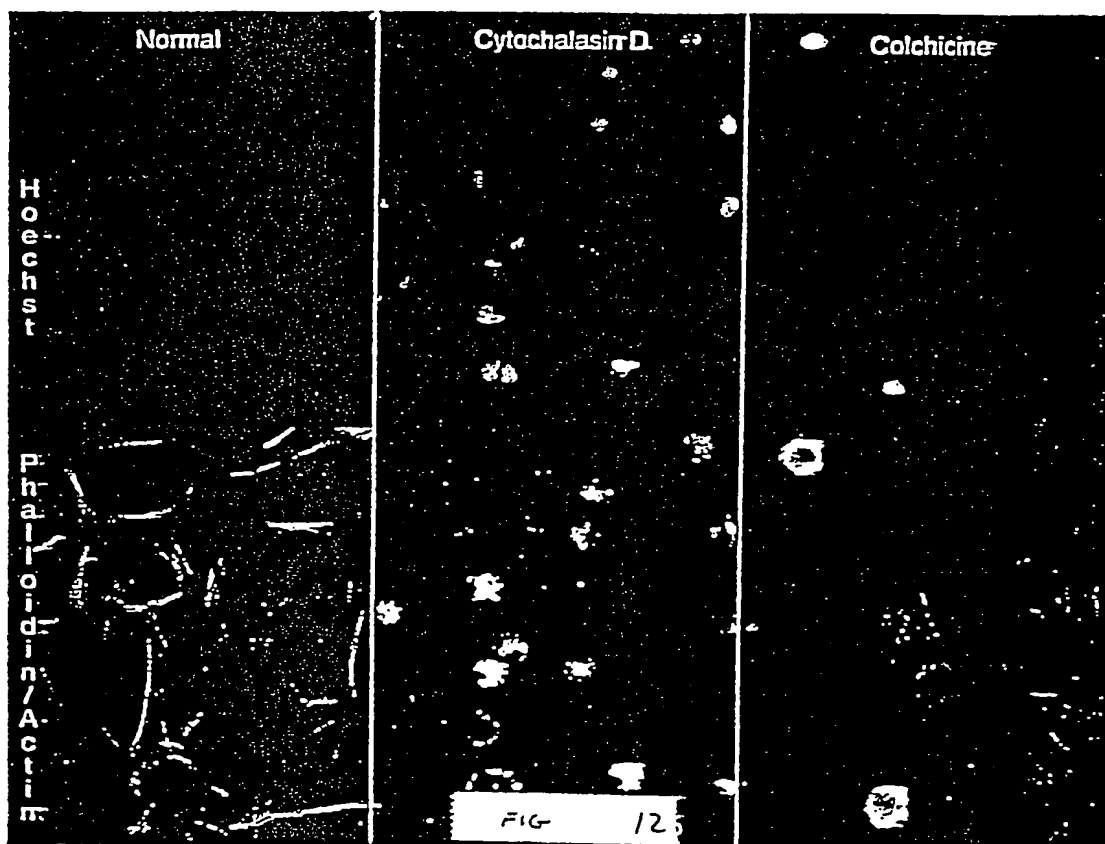


Fig. 10





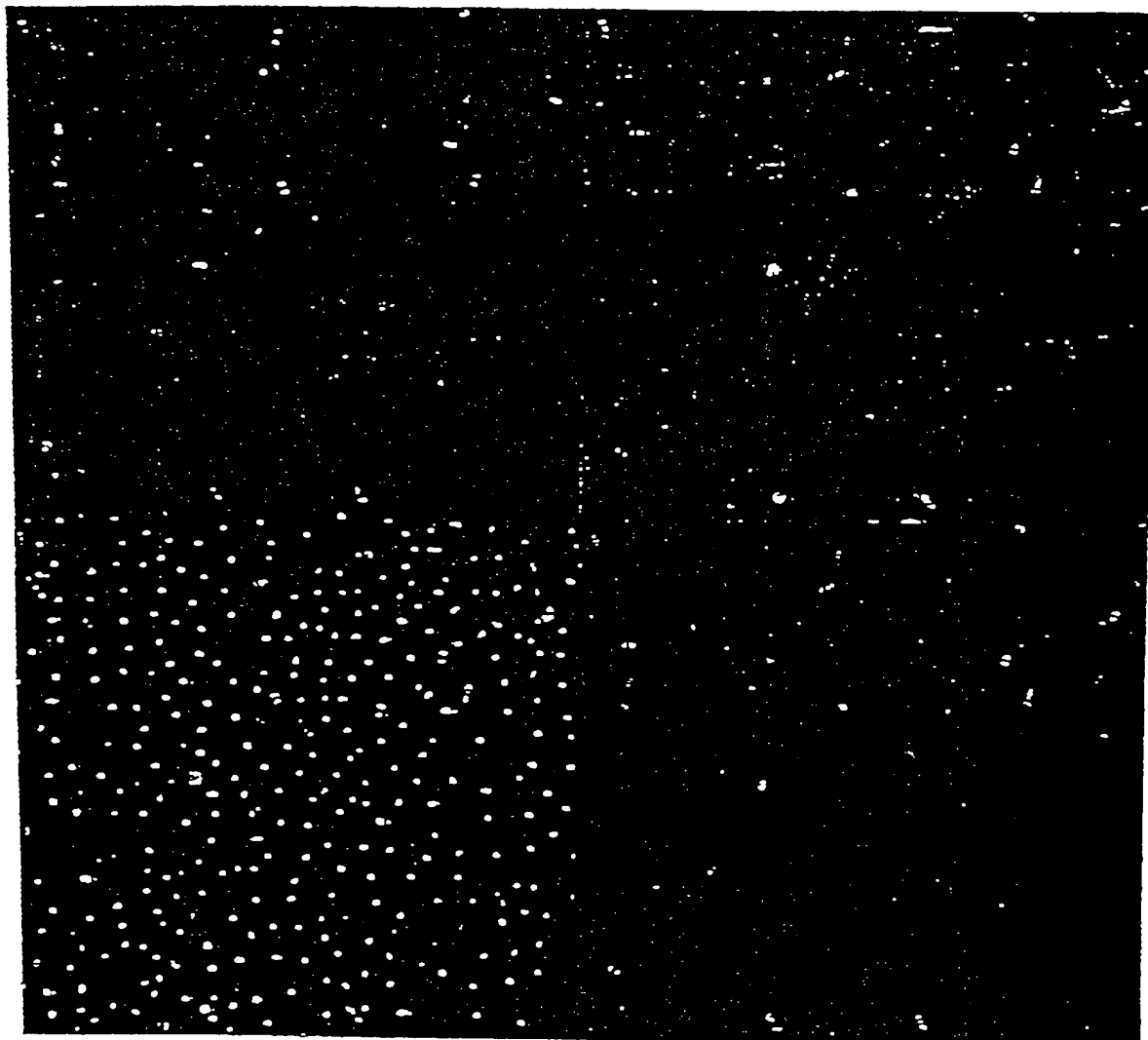


Fig 13

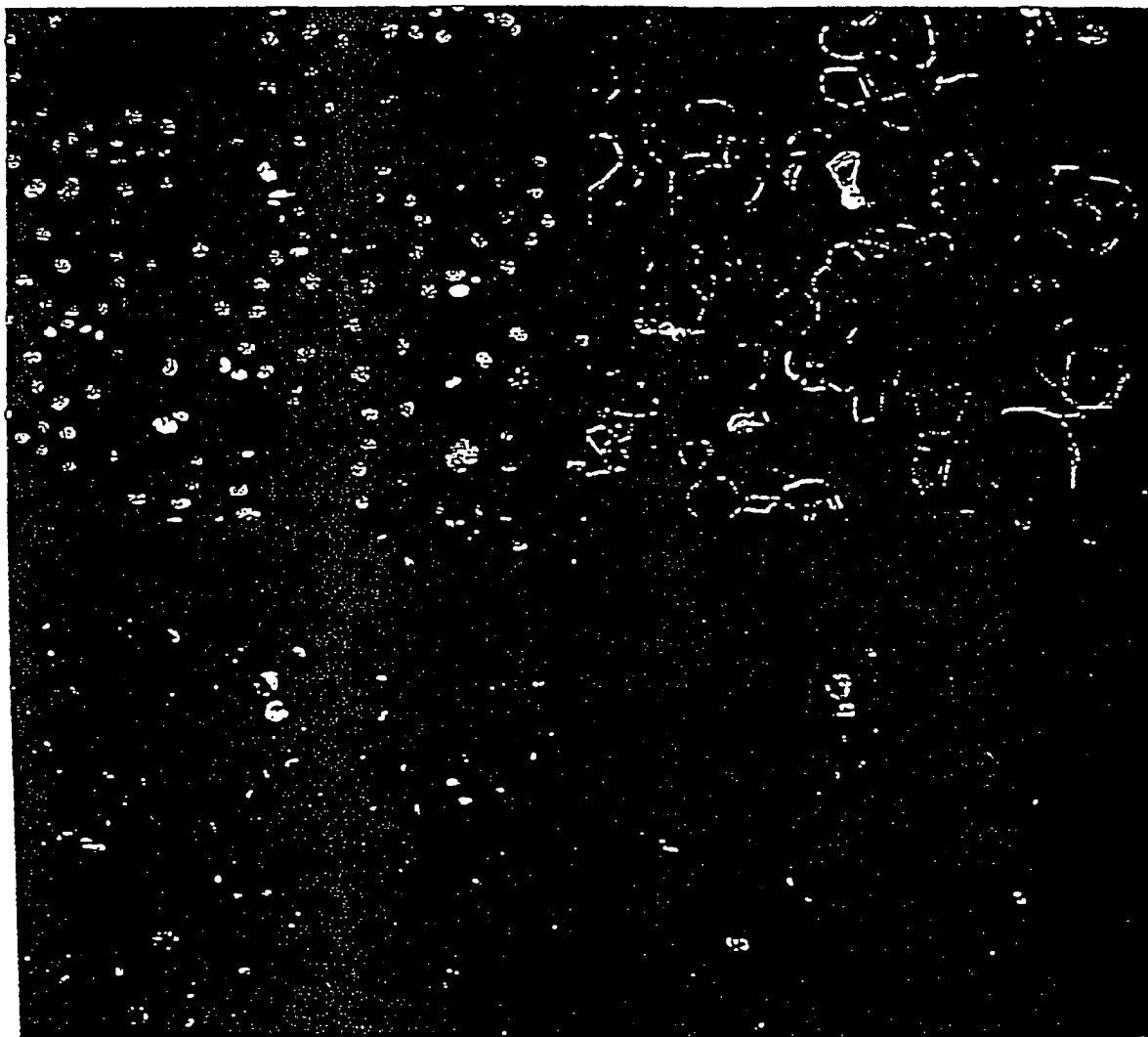


Fig 14

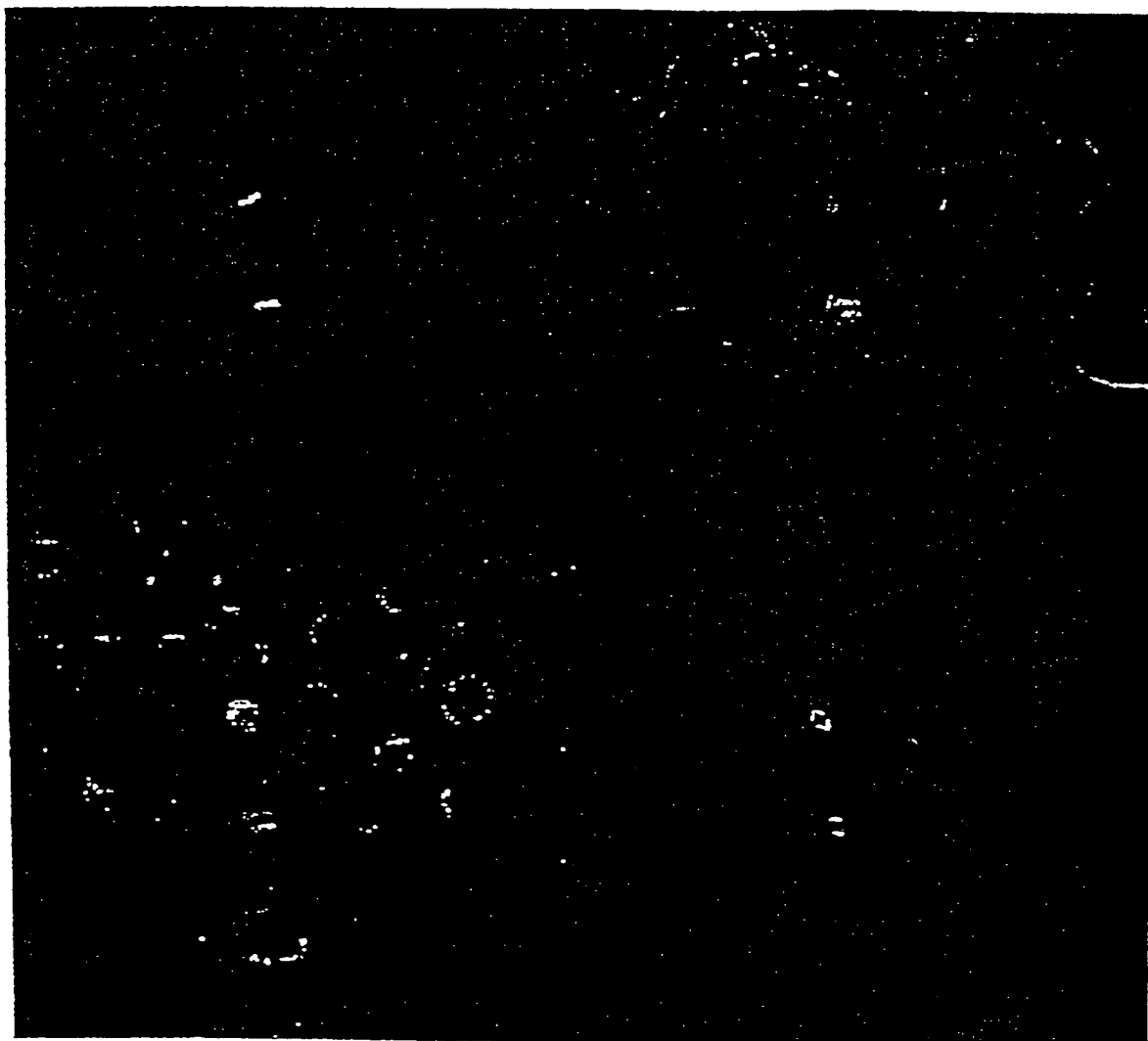


Fig 15



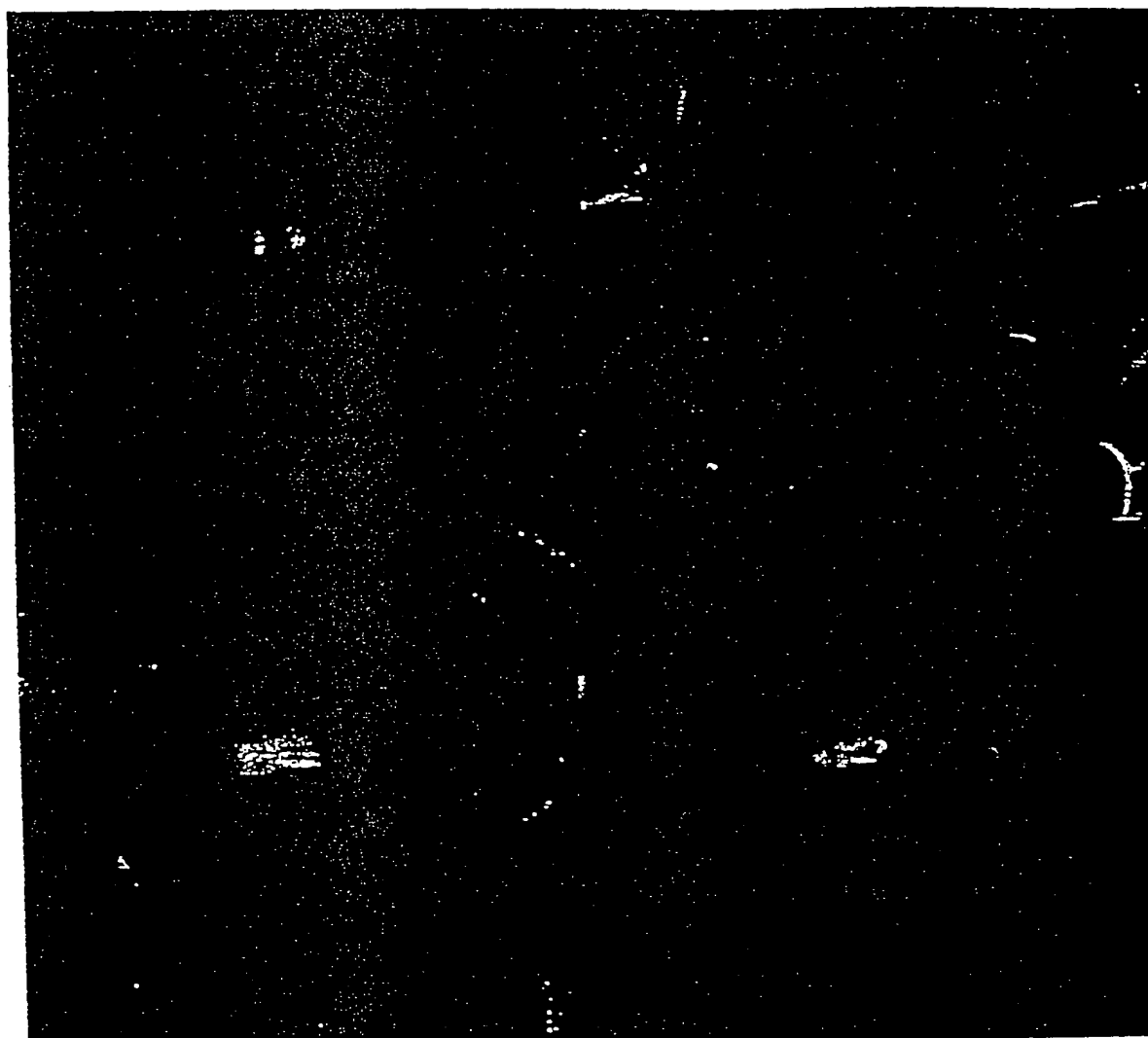


Fig 16



Fig 17

Conversion of morphometric parameters into nucleic acid code  
and clustering of the resulting sequences using Neighbor  
Joining method.

Compound:	Measurements																			
	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density
Control	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Taxol	a	t	t	t	t	t	t	t	a	t	t	t	t	t	t	t	t	t	t	t
CD	c	a	a	a	t	a	t	t	c	a	a	a	a	a	a	a	a	t	a	a
Nocodazole	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Staurosporine	g	g	c	a	a	t	a	a	t	g	a	a	a	t	g	g	g	a	a	t
Vinblastine	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	g	t	t
Hydroxyurea	g	t	t	t	t	t	t	g	t	t	t	t	t	t	t	t	t	t	c	t

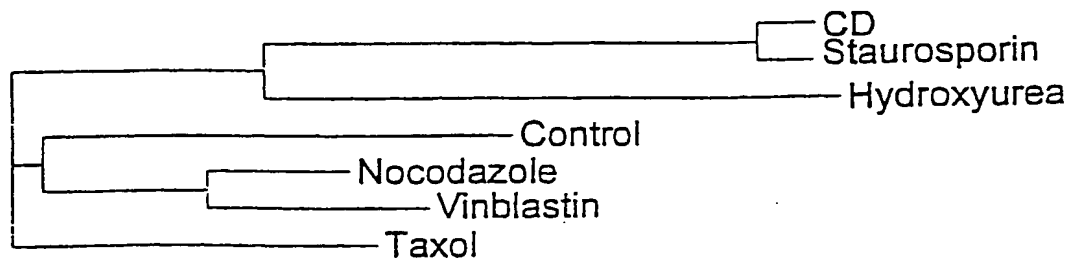


Fig 18

Conversion of morphometric parameters into amino acid codes and clustering of the resulting sequences using Neighbor Joining method.

	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Mo	IEFA Harmonic 2, Semi-	IEFA Harmonic 2, Semi-	Y
Control	H	D	T	T	N	S	D	W	M	S	T	T	T	T	C	C	D	D	M	C	T	G	T	T	Y
Taxol	G	F	M	M	P	M	P	H	G	S	M	M	W	C	T	P	T	R	C	M	M	H	M	P	S
CD	F	G	G	G	M	G	M	K	A	G	G	G	G	G	G	G	G	H	G	G	G	M	G	V	H
Nocodazole	W	F	M	M	W	M	P	T	R	S	M	M	M	F	M	W	F	M	M	R	M	M	M	F	G
Staurosporine	N	V	A	G	G	M	G	G	Y	V	G	G	G	M	V	V	V	G	G	H	G	M	G	G	V
Vinblastine	F	W	W	M	W	W	C	W	D	S	M	W	W	M	M	M	W	M	V	E	M	M	M	F	P
Hydroxyurea	S	H	H	H	H	H	H	V	H	H	H	H	H	H	H	H	H	H	H	A	H	G	H	H	D

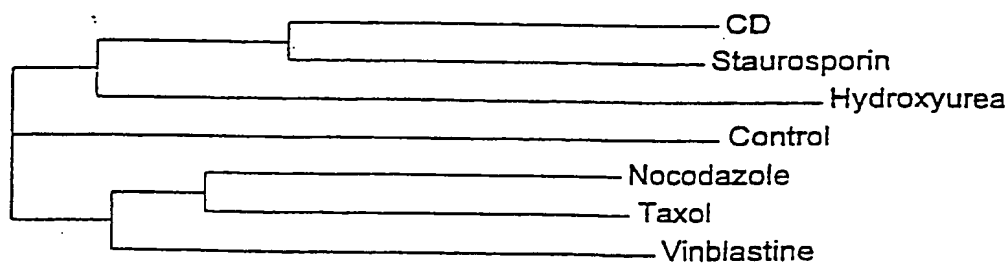


Fig 19

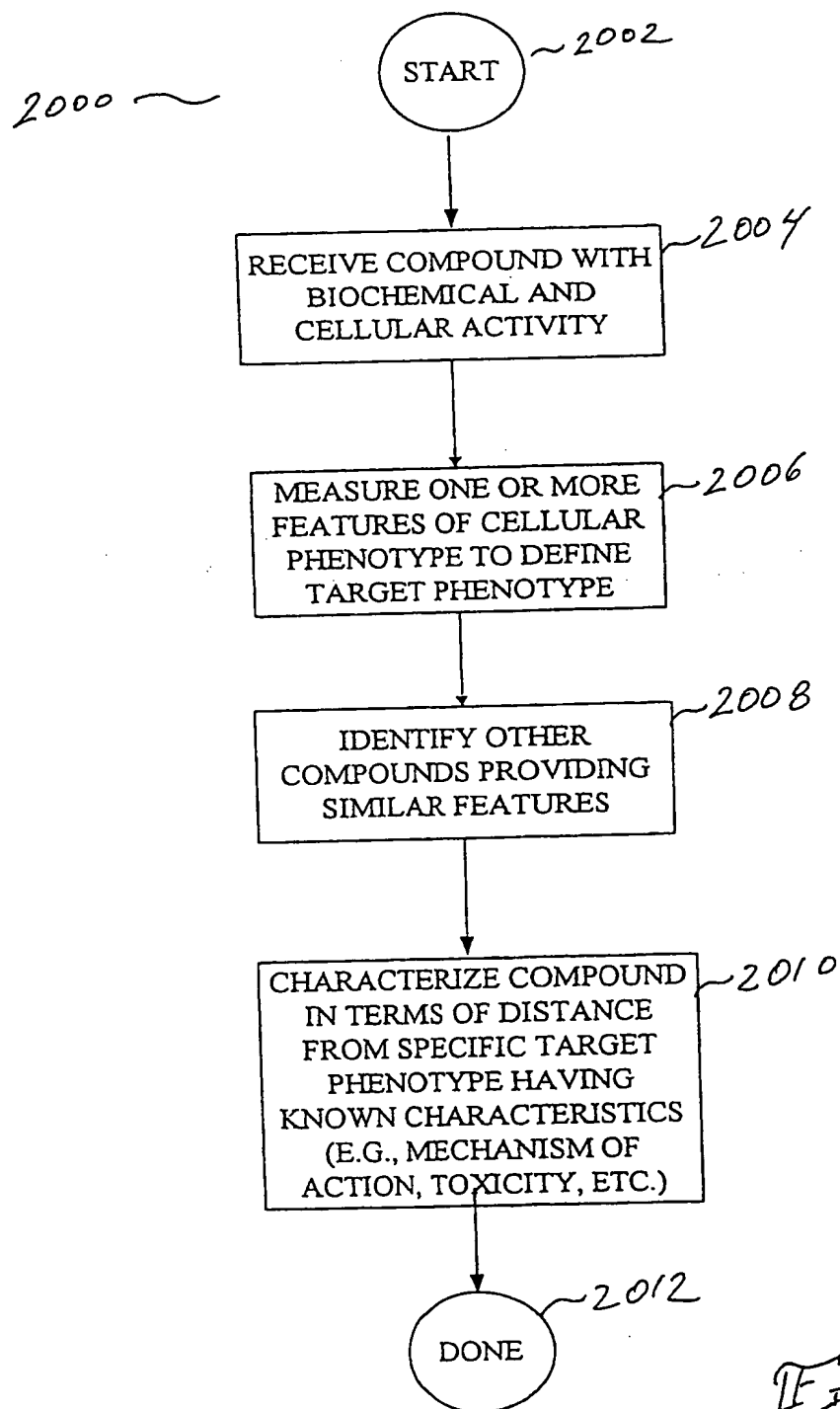


Fig 20

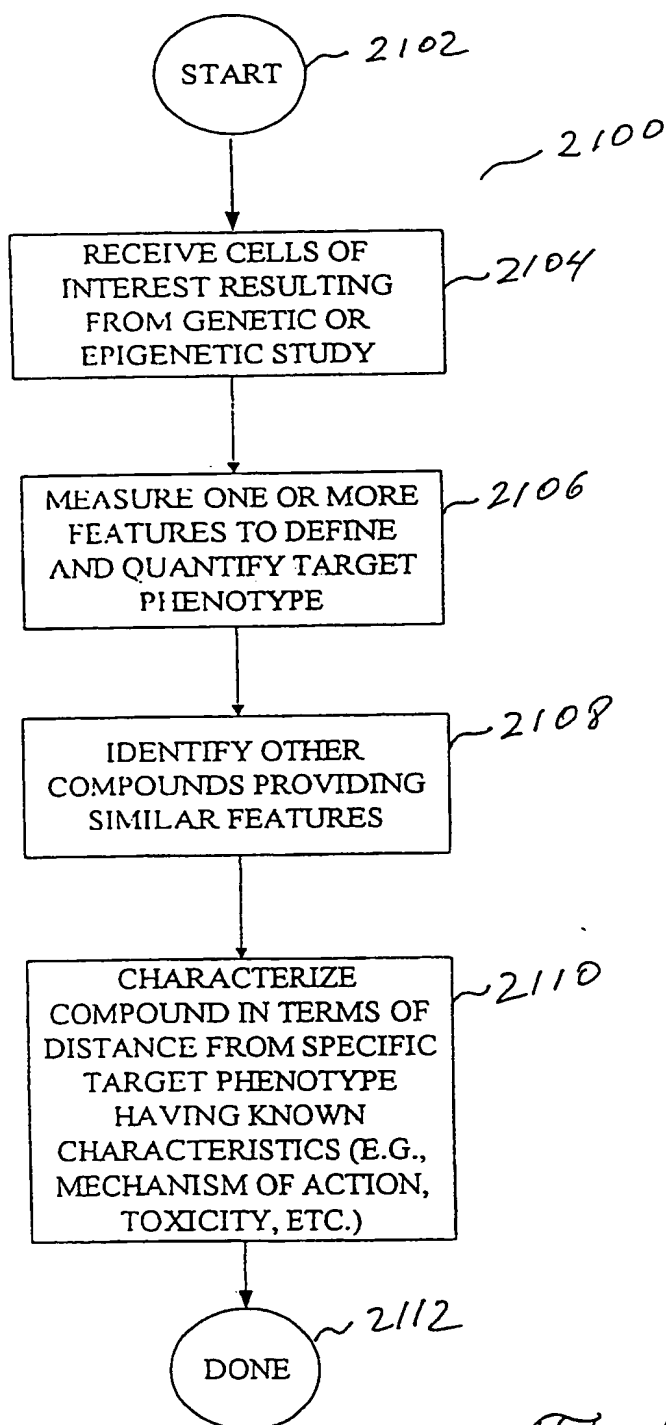


Fig 21

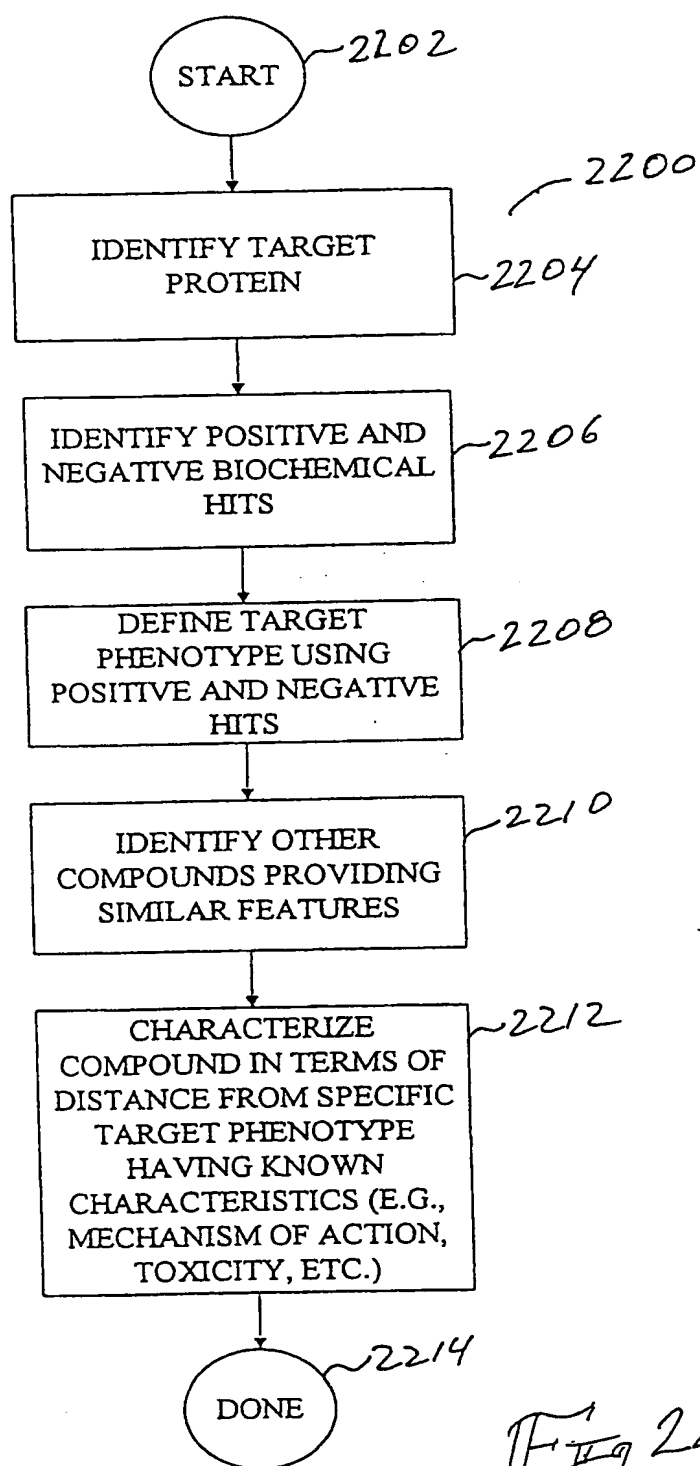


Fig 22